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THE EFFECT OF TESTOSTERONE ON CORTICOSTEROIDS IN SURGICAL TRAUMA: STUDIES IN MAN*

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ABSTRACT

The effect of pretreatment with testosterone on the adrenocortical response to surgery was studied in 5 patients undergoing subtotal gastrectomy. Testosterone propionate was administered intramuscularly or intravenously in doses of 40–200 mg. per twenty-four hours for one to five days prior to surgery. Plasma free and conjugated corticosteroid concentrations were measured before, during and following operation. The 24-hour urinary excretion of corticosteroid and ketosteroid was also measured. Five other patients who were undergoing gastrectomy but who were not pretreated with testosterone served as controls. Pretreatment with testosterone did not cause a demonstrable alteration in the concentrations of plasma free or conjugated corticosteroid nor in the urinary excretion of corticosteroid or ketosteroid, during and following operation.

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Pretreatment of normal male volunteers with testosterone failed to alter the ACTH-induced rise in plasma free and conjugated corticosteroid concentrations. Urinary excretion of corticosteroid during and following the administration of ACTH was the same in untreated volunteers as in the same subjects following pretreatment with testosterone propionate.

THE characteristic response of the adrenal cortex to surgical operation results in an elevation of the plasma concentration of corticosteroid[1] (1–5). This elevation appears to be effected by two mechanisms: first, an increased production of corticosteroid by the adrenal cortex (as measured in the adrenal vein (6, 7)) and second, an apparent delay in the disappearance rate of corticosteroid from the plasma (2, 8).

Gemzell and Notter (9) have reported that the preoperative intramuscular administration of testosterone propionate prevents the characteristic operative rise in plasma corticosteroid concentration. They also observed, as did Butler *et al.* (10), the development of a mild Addisonian crisis coincident with testosterone administration in adrenalectomized patients who had previously been maintained in good health with constant corticosteroid replacement. The authors concluded from these data that testosterone produces an alteration in the metabolism of corticosteroid in the body.

Brown and Migeon (11) also demonstrated an effect of testosterone administration on plasma corticosteroid concentration. Reece, Edwards and Jepson (12) observed the adrenocortical response of a group of patients undergoing gastrectomy who were pretreated with testosterone. They found no alteration in the urinary excretion of corticosteroid in these operative patients.

A secondary rise in plasma corticosteroid concentration following operation was observed by Christensen *et al.* (13) in adrenalectomized dogs in which the blood levels of corticosteroid had previously been maintained in a steady state by the constant infusion of hydrocortisone. This secondary rise, seemingly the result of operative trauma and anesthesia, was completely obliterated by pretreatment with testosterone. Troop (14) reported that liver homogenate from male rats reduces the side-chain of cortisone more rapidly than does the same tissue preparation from female rats of the same age. When the female animals receive testosterone, there is an enhanced rate of 17,21-dihydroxy-20-ketone side-chain reduction.

It seems apparent from these observations that the effect of pretreatment with testosterone is not the result of an altered adrenal secretory response to adrenocorticotropin (ACTH). It remains to be explained why Gemzell and Notter found no postoperative rise in the plasma corticosteroid concentration.

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[1] The term "corticosteroid" is used to mean 17-hydroxycorticosteroid.

It was of interest, therefore, to study a homogeneous group of patients undergoing similar operations under similar anesthetics, to determine the effect of testosterone propionate on the plasma corticosteroid concentration during and after operation. The testosterone propionate was given by both the intramuscular and intravenous routes.

MATERIALS AND METHODS

Ten patients (aged 45 to 64) undergoing gastrectomy and 3 healthy male volunteers (aged 22 to 28) were studied. Eight of the surgical patients were males and 2 were females. General endotracheal anesthesia was employed. Detailed data on these 10 patients are listed in Table 1.

Five of the surgical patients were pretreated with testosterone propionate according to the following protocols:

1. Intramuscular testosterone propionate in oil, 40 mg. daily for five days prior to operation—1 patient.
2. Intramuscular testosterone propionate in aqueous suspension,[2] 100 mg. daily for four days prior to, and on the morning of operation—2 patients.
3. Intravenous testosterone propionate in propylene glycol,[3] 200 mg. dissolved in 50 Gm. of human serum albumin, administered as a constant infusion (using a Bowman pump) for twelve hours preoperatively and continued for ten hours following the start of operation—2 patients.

Five of the surgical patients, the controls for this study, received no testosterone propionate. Blood samples were obtained on all 10 patients as follows: two preoperative samples were drawn between 8 a.m. and 10 a.m. on separate days. Similar samples were drawn on the day of surgery immediately prior to the induction of anesthesia and at four, six, eight and ten hours following the start of operation. Subsequent samples were drawn between 8 a.m. and 10 a.m. on the first three postoperative days. These 20-ml. blood samples were drawn into heparinized syringes. The plasma was separated by centrifugation and frozen until analyses were carried out for free and conjugated corticosteroids.

Each of the 3 healthy volunteers received an infusion of ACTH, both before and following treatment with testosterone. Two of these volunteers received aqueous testosterone propionate suspension by intramuscular injection, 100 mg. daily for four days prior to, and on the day of the ACTH test. The third volunteer received methyltestosterone orally, 400 mg. daily for three days and 200 mg. on the day of the ACTH test. The ACTH tests were carried out as follows: a 25-unit dose of ACTH (Parke, Davis & Co.) was dissolved in 1000 ml. of physiologic saline and administered as an eight-hour intravenous infusion. Blood samples for determination of plasma free and conjugated corticosteroids were withdrawn prior to, and at four, six, eight and ten hours following the start of the infusion. Urine was collected as voided by each volunteer during the eight-hour period of ACTH infusion and for two additional eight-hour periods thereafter.

The concentration of plasma free corticosteroid was determined by the method of Nelson and Samuels (15). The concentration of plasma conjugated corticosteroid was

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[2] The testosterone propionate suspension was supplied by J. E. Gajewski, M.D., Parke, Davis & Co., Detroit, Mich.

[3] The testosterone propionate in propylene glycol was prepared and supplied by James B. Lesh, Ph.D., Armour Laboratories, Kankakee, Ill.

TABLE 1. DATA ON 10 SURGICAL PATIENTS, 5 OF WHOM WERE PRETREATED WITH TESTOSTERONE

Patient No.	Hosp. No.	Sex & Age	Diagnosis	Operation	Anesthesia	Testosterone dosage	Complications
1	9L527	M 49	Benign gastric ulcer	Subtotal gastrectomy	Endotracheal—d-tubocurare, cyclopropane and ether	100 mg. testosterone propionate aqueous suspension i.m. for 4 days preop. and on day of op.	None
2	5H319	M 64	Chronic duodenal ulcer	Subtotal gastrectomy	Endotracheal—nitrous oxide and ether	219 mg. testosterone propionate in human serum albumin, i.v.	None
3	8L926	M 53	Gastrojejunocolic fistula due to marginal ulceration	Subtotal gastrectomy and closure of gastrocolic fistula	Endotracheal—nitrous oxide, ether and cyclopropane	100 mg. testosterone propionate aqueous suspension i.m. for 4 days preop. and on day of op.	None
4	83520	M 50	Marginal ulcer	Gastric resection and vagotomy	Endotracheal—cyclopropane	40 mg. testosterone propionate aqueous suspension i.m. for 5 days preop.	None
5	A2431	F 54	Chronic duodenal ulcer	Subtotal gastrectomy	Endotracheal—nitrous oxide and ether	202 mg. testosterone propionate in human serum albumin, i.v.	None
6	2J656	M 51	Chronic duodenal ulcer	Subtotal gastrectomy	Endotracheal—d-tubocurare, nitrous oxide and ether	None	Leak in duodenal stump on 7th postop. day; surgically drained; recovery
7	72602	M 54	Chronic duodenal ulcer and hiatal hernia	Subtotal gastrectomy and hiatal herniоplasty	Endotracheal—nitrous oxide and ether	None	None
8	IL645	M 59	Carcinoma of stomach	Subtotal gastrectomy	Endotracheal—d-tubocurare and cyclopropane	None	None
9	8L172	F 60	Carcinoma of stomach	Subtotal gastrectomy	Endotracheal—curare, nitrous oxide and cyclopropane	None	None
10	9L409	M 45	Chronic duodenal ulcer	Subtotal gastrectomy	Endotracheal—d-tubocurare, nitrous oxide and ether	None	Mild atelectasis and temporary ileus

determined in the following fashion: the residue after chloroform extraction of the free corticosteroid from plasma was acidified with 50 per cent acetic acid to a pH of 5.0. This mixture was then incubated for twenty-four hours at 40° C. with 1000 units per ml. of "Ketodase."[4] The hydrolyzed material was extracted three times with 1.5 volumes of freshly distilled chloroform. The chloroform extracts were combined and evaporated to dryness. The residue was partitioned between benzene and water (2:5). The benzene phase was discarded and the aqueous phase extracted three times with 1.5 volumes of chloroform. The chloroform extracts were evaporated to dryness and the residue taken up in 5 ml. of chloroform for chromatography on Florisil columns. From this point on, the procedure was identical with the method of Nelson and Samuels for the determination of free corticosteroid.

Voided urine was collected in 24-hour specimens from 3 testosterone-treated and from 3 control patients undergoing gastrectomy. This urine was analyzed for total corticosteroid content by the method of Reddy, Jenkins, and Thorn (16), as modified by Reddy (17). Total excretion of urinary ketosteroids[5] was determined by the method of Klendshoj, Feldstein and Sprague (18). The urinary creatinine content was determined by the method of Folin (19) as a check for the completeness of the 24-hour urine collections.

RESULTS

Plasma free and conjugated corticosteroid concentrations in the 5 surgical patients who were pretreated with testosterone propionate are listed in Table 2. The peak concentrations of plasma free corticosteroid occurred from six to ten hours following the start of operation. The maximum concentration of free corticosteroid in this group was from 43.9 μg. to 54.1 μg. per 100 ml. of plasma. Conjugated corticosteroid concentration reached a peak in from four to ten hours following the beginning of the operation. In 4 of the patients pretreated with testosterone propionate the maximum concentration of conjugated corticosteroid occurred at ten hours following the start of operation. The maximum concentration of conjugated corticosteroid for each patient in this group varied from 14.4 μg. to 38.9 μg. per 100 ml. of plasma.

The concentrations of free and conjugated corticosteroid in the plasma of the 5 patients who did not receive testosterone propionate also appear in Table 2. The peak concentration of plasma free corticosteroid in this group occurred from four to ten hours after the start of operation. The maximum concentration varied from 41.6 μg. to 76.2 μg. per 100 ml. of plasma. Conjugated corticosteroid concentration in the plasma of the control group reached a maximum value in from six to twenty-four hours following the beginning of operation. The maximum concentration of conjugated corticosteroid ranged from 13.3 μg. to 66.7 μg. per 100 ml. of plasma. This peak concentration of conjugated corticosteroid occurred later than did the maximum concentration of free corticosteroid in 4 of the 5 control

[4] "Ketodase" (Warner-Chilcott Laboratories) is beef liver β-glucuronidase.

[5] The term "ketosteroid" is used to mean 17-ketosteroid.

TABLE 2. EFFECT OF PRETREATMENT WITH TESTOSTERONE ON PLASMA FREE AND CONJUGATED CORTICOSTEROID CONCENTRATIONS FOLLOWING GASTRECTOMY*

Patient No.	Preoperative		Day of operation					Postoperative		
	Days		Preop.	Following start of operation				Days		
	−2	−1		4 hrs.	6 hrs.	8 hrs.	10 hrs.	1	2	3
Five Patients Pretreated with Testosterone										
1	9.6* 0	21.6 1.8	16.7 5.9	40.2 9.8	27.4 14.0	43.9 10.3	31.5 14.4	20.0 11.8	18.6 10.6	15.4 5.9
2	10.8 1.6	19.5 8.3	16.3 1.2	36.8 5.5	39.9 13.5	54.1 33.3	53.7 38.9	23.8 8.0	15.9 1.6	21.3 16.7
3	15.8 3.3	— —	3.1 0	29.4 8.0	32.3 15.7	41.7 20.8	46.9 25.2	23.4 12.4	33.9 15.6	— —
4	11.9 0.7	— —	3.8 4.4	47.8 14.9	48.5 11.5	37.5 14.6	34.8 10.5	25.3 9.3	21.9 6.8	17.0 6.0
5	10.0 8.3	15.8 12.3	11.0 6.5	38.1 20.2	43.7 27.1	46.6 23.1	43.9 33.7	22.9 23.6	— 14.6	11.6 9.8
Five Patients Not Treated with Testosterone										
6	14.4 7.8	8.5 0	5.5 0	41.6 19.2	36.9 17.8	41.3 29.2	31.2 33.5	33.8 22.7	— —	28.2 10.0
7	10.3 0	4.3 0	14.6 0	30.0 16.7	34.3 24.1	41.1 42.7	46.8 53.6	28.6 66.7	30.6 24.1	20.3 21.3
8	— —	5.7 6.5	8.7 2.7	38.3 20.8	47.5 20.2	49.7 34.5	76.2 16.3	32.0 21.8	— 14.3	18.0 7.6
9	8.1 5.1	11.3 0	7.9 6.3	44.5 23.8	44.2 28.5	41.0 22.7	43.1 25.0	29.6 12.5	— —	— —
10	— —	10.8 2.2	22.9 2.6	55.6 10.3	— —	54.5 13.3	36/5 —	23.1 0	— —	— —

* For each patient, the upper line of values represents free plasma corticosteroid (μg. per 100 ml.) and the lower line, conjugated corticosteroid.

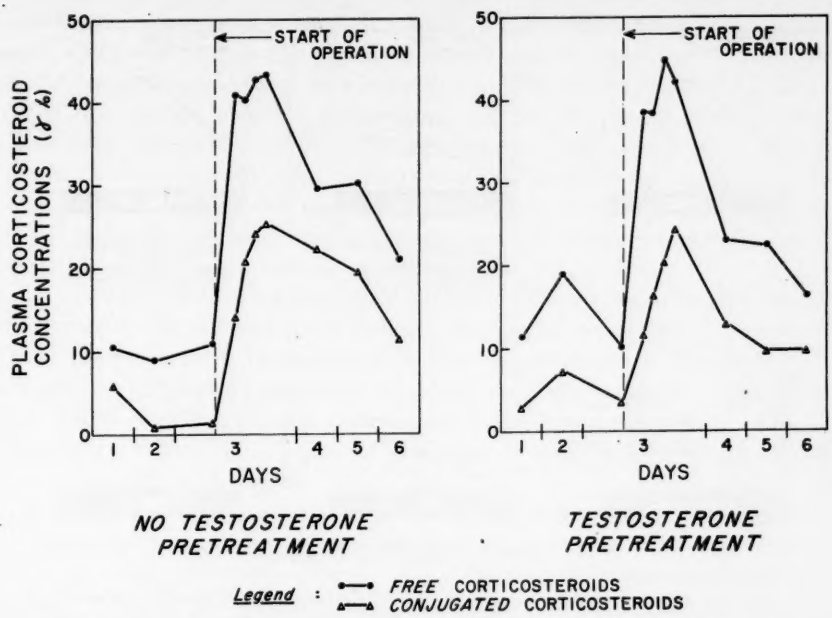

FIG. 1. The effect of testosterone on plasma free and conjugated corticosteroid concentrations (μg. per 100 ml.) following gastrectomy. The values on the left are the mean plasma corticosteroid concentrations in 5 untreated control patients who did not receive testosterone prior to gastrectomy. The values on the right are the mean plasma corticosteroid concentrations in 5 patients who received testosterone propionate prior to gastrectomy.

patients. The mean plasma free and conjugated corticosteroid concentrations for the pretreated as well as the control patients are graphically presented in Figure 1.

There was no significant alteration from the control series in the postoperative excretion of corticosteroid and ketosteroid in the urine of patients pretreated with testosterone propionate, although there was the expected increase in urinary excretion of ketosteroid on the day of operation, during the administration of the testosterone.

Pretreatment of the normal volunteers with testosterone produced no consistent alteration in the ACTH-induced rise in plasma free and conjugated corticosteroid concentrations. Figure 2 shows the plasma corticosteroid levels during ACTH stimulation in the normal volunteers, before and after treatment with testosterone. The urinary excretion of corticosteroid likewise was unaltered by pretreatment of the subjects with testosterone.

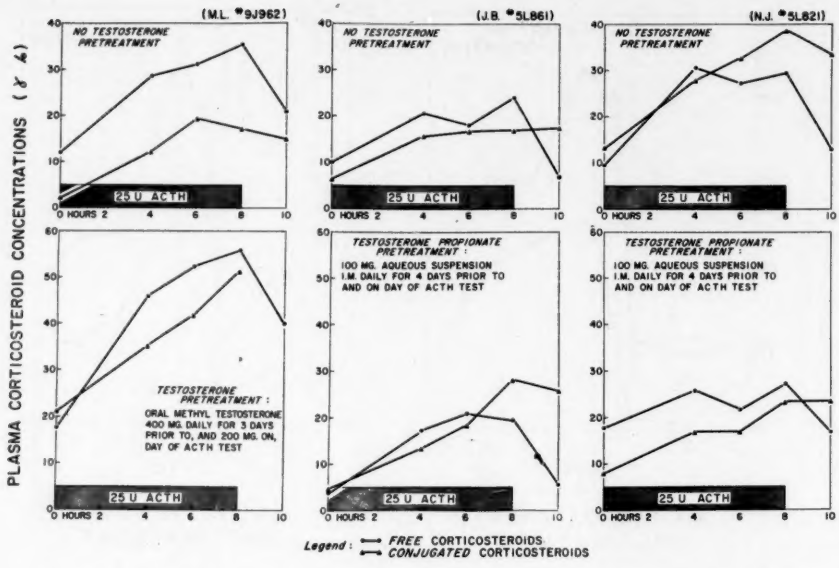
(M.L. *9J962)
(J.B. *5L861)
(N.J. *5L821)
NO TESTOSTERONE PRETREATMENT
25 U ACTH
0 HOURS 2 4 6 8 10
PLASMA CORTICOSTEROID CONCENTRATIONS (γ %)
TESTOSTERONE PROPIONATE PRETREATMENT: 100 MG. AQUEOUS SUSPENSION I.M. DAILY FOR 4 DAYS PRIOR TO AND ON DAY OF ACTH TEST
TESTOSTERONE PRETREATMENT: ORAL METHYL TESTOSTERONE 400 MG. DAILY FOR 3 DAYS PRIOR TO, AND 200 MG. ON, DAY OF ACTH TEST
Legend: FREE CORTICOSTEROIDS
CONJUGATED CORTICOSTEROIDS

FIG. 2. The effect of testosterone on the ACTH-induced rise in plasma free and conjugated corticosteroid levels (μg. per 100 ml.) in 3 healthy volunteers before and following treatment with testosterone as indicated.

DISCUSSION

Pretreatment with testosterone propionate of patients undergoing gastrectomy did not cause any pronounced alteration in the pituitary-adrenal response to operation, as measured by the concentrations of plasma free and conjugated corticosteroid. In 8 of 10 patients the results were uniform; however, in 2 patients of the control group, the plasma corticosteroid values were abnormal. Patient #7 had an unusually high level of conjugated corticosteroid during operation and on the first postoperative day. This might be attributed to a difficult gastrectomy in association with an esophageal hiatus hernioplasty. Helmreich *et al.* (4) reported a patient in whom the conjugated corticosteroid values also exceeded the free values. They were unable to explain this finding, but emphasized the marked variation in plasma conjugated corticosteroid concentrations following operation. Our Patient #8 demonstrated a high plasma free corticosteroid level ten hours following the start of operation; this single high value was unexplained.

Thus, our findings do not corroborate those of Gemzell and Notter, nor do these observations in man corroborate our somewhat analogous experiments in the dog (13). The operative procedures in the study of Gemzell

and Notter were not described, and some of the patients may have undergone operations that do not regularly cause a significant increase of adrenocortical secretion. No mention was made by them of the type of anesthetics used. The adrenal response to operation may be modified by the type of anesthesia (20, 21). Since blood samples were obtained by them at the completion of operation and not again until the following morning, it is probable that some of the corticosteroid peaks were missed, since considerable variation has been observed in the timing, peak concentration, and duration of the postoperative corticosteroid response (3, 5).

In our study, no lasting alteration in the urinary excretion of corticosteroid and ketosteroid was noted in the testosterone-treated patients undergoing gastrectomy as compared with the control patients. Variability of the urinary ketosteroid excretion following operation was again noted (22). The sizable dosage of intramuscular and intravenous testosterone propionate used did produce a transient elevation in urinary ketosteroid excretion on the day of operation.

Pretreatment with testosterone caused no consistent change in the plasma free and conjugated corticosteroid response to ACTH stimulation in the normal volunteers. The urinary excretion of corticosteroid in response to ACTH likewise remained unchanged following the administration of testosterone. Other investigators have also been unable to demonstrate a testosterone effect on the ACTH-induced rise in plasma corticosteroid concentration in normal subjects (9, 11).

In the present study, there was no evidence that testosterone modifies the pituitary-adrenal response to surgical trauma. This response, as measured by the plasma concentration and urinary excretion of corticosteroid, appeared to be similar in both the testosterone-treated and control groups of subjects. There was also no modification of the characteristic delay in the rates of metabolism and disposal of corticosteroid in the patients undergoing operation. If testosterone can enhance the rate of intermediary metabolism of corticosteroid, we were unable to demonstrate it by the methods used in this study.

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URINARY STEROIDS IN THE HYPERTENSIVE FORM OF CONGENITAL ADRENAL HYPERPLASIA*†

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ABSTRACT

Four patients with the hypertensive form of virilizing adrenal hyperplasia were studied and compared with 6 patients with the simple virilizing type, and 7 others with the salt-losing type of this syndrome. The urinary patterns of excretion of total 17-ketosteroids, pregnanetriol, tetrahydrocortisol, tetrahydrocortisone, tetrahydro-S and tetrahydrodesoxycorticosterone were compared in each type of manifestation of this syndrome. The results show a significant variation in the degree of enzymatic defects present, with the majority of patients demonstrating an ability to perform the sequential enzymatic processes of hydroxylation necessary for the synthesis of cortisol. Prolonged stimulation with ACTH did not significantly increase this ability. In 2 siblings with the hypertensive form of the syndrome, clinical manifestation appeared only after puberty, indicating that this disorder may be a cause of acquired hypertension in the adult. The rarity of this abnormality is indicated by the negative results in a survey of a large hypertension clinic and a female hirsutism clinic. Values for urinary 17-ketosteroids and pregnanetriol may be misleading in the diagnosis of the hypertensive adrenogenital syndrome, for these may be within normal ranges even though virilization and hypertension are progressing. Fractionation of the urinary corticosteroids was necessary to prove the diagnosis in 2 cases.

INTRODUCTION

THE clinical syndrome of congenital adrenal hyperplasia accompanied by hypertension has been well defined in previous reports from this clinic and elsewhere (1–4). Wilkins and associates pointed out that the variations in symptomatology in cases of congenital adrenal hyperplasia

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‡ Recipient of a research fellowship of The United States Public Health Service (HF-8854).

suggest that there may be a number of different patterns of abnormal adrenal activity (2). In recent years, extensive studies of the steroids produced in this syndrome have confirmed the clinical impression that biochemical variations do exist. This has been most clearly demonstrated in the differentiation of the hypertensive form of the syndrome from the nonhypertensive forms (4, 5). Cases of postpubertal virilism in females have recently been reported by Kappas *et al.* (6) and Bush and Mahesh (7) with thorough investigation of the patterns of excretion of urinary 17-ketosteroids. In this clinic, 2 patients have been observed who became virilized after puberty and who also acquired hypertension after puberty. The fact that they were siblings suggests an hereditary defect. Studies were undertaken of the steroidal patterns[1] in 4 patients with hypertension and virilization to determine if they were truly identical with those in the 3 cases already reported in the literature (4, 8, 9). For the purposes of comparison, the same investigative techniques were utilized in studying 6 cases of simple virilization due to congenital adrenal hyperplasia and 7 cases of the "salt-losing" type of this syndrome.

CASE REPORTS

Case I

J. E. (infant female—HLH #B-55941). This Negro child was first brought to the Harriet Lane Home at the age of 7 months. An enlarged clitoris had been noted at birth, and the enlargement had been progressive. There had been no illnesses. On physical examination she was found to have a blood pressure of 130/90 mm. Hg in the arm and 145/100 mm. in the leg. She was 31 inches in length and weighed 19½ pounds. The phallus was enlarged, measuring 2.8 by 1.2 cm. There was only a mild degree of labioscrotal fusion posteriorly. No sexual hair was present and no gonads were palpable in the labioscrotal folds. A cervix was palpable on rectal examination. The bone age was 1 year. A buccal smear showed a chromatin positive pattern in the cells. Urinary 17-ketosteroid excretion, determined on five successive days, was 0.7, 0.8, 0.3, 0.5 and 0.3 mg. per twenty-four hours. Urinary pregnanetriol excretion was 0.08 mg. per twenty-four hours. The concentration of serum urea nitrogen was 10 mg. per 100 ml., sodium 149 mEq. per liter, potassium 5.0 mEq. per liter, and chloride 107 mEq. per liter. Owing to the relatively low levels of ketosteroids, a more detailed search for a cause of hypertension was undertaken. An intravenous pyelogram, aortogram and renal arteriogram were normal. Urinary 17-hydroxycorticosteroid excretion was 5.3 mg. per twenty-four hours. Because this was considered unusually high, fractionation of these corticoids was performed

[1] The following abbreviations will be used for standard nomenclature of the steroids discussed:

Tetrahydro-S (THS)—pregnane-3α,17α-21-triol-20-one.
Tetrahydrocortisol (THF)—pregnane-3α,11β,17α,21-tetra-ol-20-one.
Tetrahydrocortisone (THE)—pregnane-3α,17α,21-triol-11,20-dione.
Tetrahydrodesoxycorticosterone (TH-DOC)—pregnane-3α,21-diol-20-one.
Pregnanetriol—pregnane-3α,17α,20α-triol.
Cortisol—Δ^4-pregnene-11β,17α,21-triol-3,20-dione.

TABLE 1. QUANTITATIVE DETERMINATION OF URINARY STEROIDS IN 3 TYPES OF CONGENITAL ADRENAL HYPERPLASIA

	Age	Sex	Urinary steroids (mg./24 hrs.)*							Remarks
			P-triol	THS	THE	THF	THE +THF	TH-DOC	17-KS	
Hypertensive type										
A.A.	47 yrs.	F	1.1	3.3	—	—	0.6	0.8	2.9	Control
(Case IV)			11.2	68.8	—	—	8.2	0.6	20.0	Day 2, ACTH test
J.J.	29 yrs.	F	2.0	22.4	1.1	3.3	4.4	0.2	4.2	Control
(Case III)			2.2	49.3	1.1	3.1	4.2	1.5	3.9	Day 3, ACTH test
J.E.	1 yr.	F	0.1	12.5	—	—	1.4	0	1.5	Control
(Case I)			0.2	26.3	—	—	1.6	0.1	1.8	Day 3, ACTH test
J.F.	8 yrs.	M	7.7	67.5	0	0	0	1.0	11.7	Control
(Case II)			16.9	70.8	0	0	0	2.8	10.2	Day 3, ACTH test
Simple virilizing type										
V.A.	8 mos.	F	3.1	0	—	—	0.4	0	2.7	Control
			4.7	0	—	—	0.3	0	3.5	Day 3, ACTH test
C.U.	9½ mos.	F	0.1	0	1.2	0.2	1.4	0	5.2	Control
			11.0	0	1.3	0.3	1.6	0	6.9	Day 3, ACTH test
S.W.	4 $\frac{10}{12}$ yrs.	F	7.5	0	0.8	0.3	1.1	0	6.4	Control
			11.7	0.1	3.2	0.7	3.9	0	6.2	Day 3, ACTH test
K.M.	6 yrs.	M	16.4	0	1.6	0.9	2.5	0	22.8	Control
			42.6	0	2.4	0.5	2.9	0	32.6	Day 3, ACTH test
D.B.	14 yrs.	M	33.0	0	3.2	0.9	4.1	0	38.7	Untreated, 18 mos.
J.R.	22 yrs.	F	17.9	0	1.5	0.3	1.8	0	52.0	Untreated
Salt-losing type										
K.S.	2 wks.	F	0.8	0	—	—	0.8	0	5.6	Untreated
E.S.	2 wks.	F	0.8	0	0	0	0	0	3.5	Untreated
J.S.	2 wks.	F	0.3	0	0	0	0	0	4.3	Untreated
D.H.	3 wks.	F	1.9	0	0	0	0	0	5.8	Untreated
L.L.	4 wks.	F	0.7	0	0	0	0	0	4.2	Untreated
R.H.	8 mos.	F	7.9	0	0	0	0	0	3.9	Untreated
W.W.	2½ yrs.	F	46.3	0	—	—	0.6	0	6.0	Untreated; salt-deprived

* P-triol = pregnanetriol; TSH = tetrahydro-S; THE = tetrahydrocortisone; THF = tetrahydrocortisol; TH-DOC = tetrahydrodesoxycorticosterone; 17-KS = 17-ketosteroids.

on paper and the greater part of this material was found to correspond to tetrahydro-S.

The child did not return for a five-month interval, and during this time progressive virilization had occurred. The height was now 33½ inches, and the weight 26 pounds. Blood pressure was 150/90, and the pulse 130 per minute. Four lower and two upper incisors had appeared. About two dozen dark coarse pubic hairs were apparent and the clitoris had enlarged further. The bone age had advanced to the 3-year level. The child was hospitalized for further study. Urinary 17-ketosteroid excretion now ranged from 1.4 to 2.1 mg. per twenty-four hours, with an average of 1.5 mg. There was no significant response to administration of ACTHAR-gel for three days. Urinary 17-hydroxycorticosteroid excretion was 14 mg. per twenty-four hours and rose to 28 mg. with ACTH stimulation. Fractionation showed the greatest portion of these corticosteroids to be tetrahydro-S. Results of the complete study are shown in Table 1. When the child was

discharged she was receiving triamcinolone for suppression therapy. The blood pressure has remained between 100 and 114 mm. systolic and 80 to 90 mm. diastolic.

Case II

J. F. (male, born February 15, 1950—HLH #B-4449). The parents of this boy treated his condition very casually and medical advice was not sought until he was 3½ years of age. The mother had had severe edema and moderate hypertension during pregnancy. The child was born at term, weighing 8 pounds 15 ounces. Immunizations had been neglected. Accomplishments were normal but growth had been excessive from the time of birth. The mother thought that there had been a large penis at birth and that there had been progressive enlargement. Erections had been frequent. Pubic fuzz had appeared at the age of 2 years and a deep voice developed at age 2½ years. Since then, his growth had proceeded rapidly and his appetite was described as huge.

At the time of examination at age 3½, his height age was 7 years (47.3 inches) and he weighed 53¾ pounds. His blood pressure was 130/88 and pulse 92. He was deeply pigmented and acne was present on the face. The testes were of infantile size, but the scrotum and penis were of mature size. A prostate was palpable. He had 20 deciduous teeth. The bone age was 11 years. There was no hypertensive retinopathy. The intelligence quotient was reported as 140. A thorough "hypertensive" work-up yielded normal findings. Blood chemical values were normal. Results of the Fishberg, phenolsulfonphthalein (P.S.P.) excretion and benzodioxane tests were not abnormal. An intravenous pyelogram, an electrocardiogram and a ballistocardiogram were interpreted as normal. Glucose tolerance and insulin tolerance curves were also normal. Urinary steroid studies confirmed the diagnosis of congenital adrenal hyperplasia. Urinary 17-ketosteroids ranged from 11.4 mg. to 20.7 mg. per twenty-four hours. Formaldehydogenic steroid excretion on one occasion was 1.14 mg. per twenty-four hours. ACTH was given intramuscularly for three days, but 17-ketosteroid excretion did not increase and ranged from 8.1 to 16.3 mg. per twenty-four hours. Formaldehydogenic steroids on the third day measured 1.42 mg. Pregnanetriol excretion was 3.3 mg. per twenty-four hours.

Cortisone suppression therapy caused a fall in 24-hour urinary 17-ketosteroids to a range of 0.8–4.5 mg. Blood pressure fell after fourteen days of treatment to 104/66. The maintenance dosage of cortisone was 37.5 mg. every three days intramuscularly. With this regimen, adrenocortical secretion was kept well suppressed for three years. Pigmentation decreased markedly, and 24-hour urinary 17-ketosteroids never exceeded 3.3 mg. The bone age advanced to 12½ years and the testes began to enlarge. His height increased to 55½ inches.

The parents failed to return to the clinic for two years. Cortisone therapy became quite irregular and apparently was discontinued entirely for approximately six months before the family was again located. The boy was then 8 years old and had become deeply pigmented, with a blood pressure of 130/80. Height had increased to 58¾ inches (height age, 12½ years) but the bone age remained 12½ years. The 24-hour urinary excretion of 17-ketosteroids was 6.1 to 13.7 mg.; of 17-hydroxycorticosteroids, 73.5 mg.; and of pregnanetriol, 7.7 mg. An intravenous ACTH test produced no significant changes in the serum level of hydroxycorticosteroids (initial level, 38.6 μg.; two hours, 34.0 μg.; four hours, 32.5 μg.; and six hours, 41.0 μg.). ACTH-gel was given for two subsequent days, and on the last day the urinary 17-hydroxycorticosteroids measured 80.9 mg. Urinary 17-ketosteroids during the three days of ACTH administration ranged between 6.9 and 10.2 mg. per twenty-four hours. Fractionation of the urinary corticosteroids was carried out at this time and the results are listed in Table 1.

After cortisone suppression therapy was reinstituted, the urinary 24-hour excretion of 17-ketosteroids fell to between 1.5 and 4.8 mg. After an uneventful tonsillectomy, the patient was discharged. At this time, medication was oral cortisone in a dosage of 12.5 mg. three times daily.

Case III

J. J. (white female aged 29 years—JHH #579470). This patient was first seen at Johns Hopkins Hospital at the age of 22 years. According to the history, she had grown and developed normally until the age of 6 years. At that time she underwent a growth spurt, and breasts and pubic hair developed. Menstruation started within the next year and occurred regularly every twenty-eight days. By the age of 7 years she was fully grown to her present adult height. Information obtained from her medical history at the age of 7 showed her blood pressure to have been 140/80 on repeated occasions. She had begun to complain of occasional headaches. When she was 10 years old, menstruation ceased, but female sex characteristics remained unchanged. At the age of 15½, menses reappeared and had continued monthly thereafter. Headaches had ceased and she did not seek medical advice for the next five years. She married at age 18 and experienced unusually marked libidinous desire. By the age of 20, she noticed that she was becoming hirsute on the face and extremities and that pubic hair was beginning to extend up onto the abdomen. Headaches recurred and her physician found that she had severe hypertension. During the next two years, hypertension increased to 212/134 and she was referred to this hospital for study.

On physical examination in 1951 she was a 22-year-old girl who was 57 inches tall and weighed 101 pounds. The breasts were well developed and the vaginal mucosa and labia minora were well estrinized. Musculature was well developed and the clitoris was slightly enlarged, but there was no labial fusion. Pubic hair was abundant and extended onto the abdomen to the umbilicus. Facial hair was present on the cheeks and required shaving. A moderate growth of hair on the upper lip did not require shaving. Arms and legs were conspicuously hirsute.

Laboratory findings included a normal urine (routine analysis), normal P.S.P. excretion, a normal intravenous pyelogram, and a normal level of serum nonprotein nitrogen (28 mg. per 100 ml.). Results of the serologic test for syphilis were negative. X-ray examination of the skull showed a normal sella. The level of serum cholesterol was 185 mg. per 100 ml.; sodium, 141 mEq. per liter; potassium 4.7 mEq. per liter; chlorides, 103 mEq. per liter; calcium, 10.2 mg. per 100 ml.; and phosphorus, 3.8 mg. per 100 ml. Urinary 17-ketosteroids ranged from 24 to 40 mg. per twenty-four hours, with an average of 33 mg. The 24-hour excretion of "reducing lipids" was reported as 3.32 mg. on one occasion (normal, 0.2 to 0.8 mg.). Cortisone suppression studies ruled out the presence of a tumor, and urinary 17-ketosteroids dropped to 5 mg. (average) per twenty-four hours after nine days of treatment. The blood pressure dropped to 140/100 and did not return to elevated levels.

For the next six and a half years, she was maintained with oral cortisone, 12.5 mg. three times a day. Ketosteroids were adequately suppressed and normal blood pressure was maintained. Hirsutism remained a mild problem but was easily controlled by cosmetic methods. In August 1958, cortisone therapy was discontinued; five months later she was hospitalized for study. During the five-month period of withdrawal, her blood pressure had gradually risen to 190/150 with recurrence of headaches and an increase in hirsutism. However, urinary 17-ketosteroid excretion had remained low, never exceeding 5.6 mg. per twenty-four hours. ACTHAR-gel was administered in a dosage of

40 units twice daily for three days. The results of urinary steroid fractionation studies are listed in Table 1. The patient was then discharged. Steroid therapy was reinstituted, and the blood pressure returned to normal levels.

Case IV

A. A. (white female aged 47 years—JHH #826384). This woman was requested to appear for examination because of the similarity of her symptoms to those of her sister (Case III). Her childhood growth and development were described as entirely normal. Menarche had occurred normally at the age of 12 years. At the ages of 14 and 16 years she had been hospitalized for periods of two weeks for "nervous breakdowns." Excessive facial and body hair had begun to appear at this time. She had married at the age of 17 years and had never become pregnant. Menstrual periods were quite regular from the age of 12 to the age of 36 years, when a dilation and curettage operation was performed for acquired menorrhagia. Menses ceased thereafter and she had had no menstrual periods for eleven years. She was known to have had hypertension since the age of 20 years but had not been treated until age 40, when various antihypertensive drugs had been tried without effect. Systolic blood pressure had ranged from 200 to 280 mm. Hg.

Six months before being asked to come to this clinic, she had requested her physician to treat her with cortisone, since she had become aware that this drug was helping her sister. Unfortunately, no initial studies were performed. She was given oral cortisone, 25 mg. three times daily, for three months and then the dosage was changed to 25 mg. twice daily. After four months she experienced a pink menstrual discharge lasting one day. The blood pressure was reported to have decreased to 144 mm. Hg systolic, and she thought that the hirsutism had decreased slightly. She complained of fullness and tenderness of the breasts for one month.

At the time of physical examination the blood pressure was 175/110, mm. Hg, and the pulse 110 beats per minute. She was 60 inches tall and weighed 145 pounds. Moderate obesity was present but the musculature was not abnormally developed for a female. There were no striae or bruises and her appearance did not suggest Cushing's syndrome. Breasts and nipples were well developed, with a few scattered black hairs on the breasts. There was abundant axillary hair, a heavy growth of facial hair on the cheeks and upper lip (requiring shaving daily) and a heavy growth of coarse black hair on the arms and legs. Pubic hair was abundant and extended onto the abdomen. There was bitemporal regression of the scalp hair but no crown baldness. The clitoris was not abnormally enlarged, there was no labioscrotal fusion and the introitus was normal. The cervical-uterine ratio was described as that of a "normal adult." Ovaries could not be felt on pelvic examination. There were no adnexal masses and the cul-de-sac was free of masses. The only other noteworthy finding was that funduscopic examination showed a moderate narrowing and tortuosity of the vessels with no nicking and no exudates or hemorrhages.

Urinary 17-ketosteroids. While the patient was receiving cortisone the 24-hour excretion of 17-ketosteroids was 3.6 mg. and 2.5 mg. on two separate days. For the purpose of investigation, cortisone was discontinued. After two weeks, the blood pressure had risen to 192/112, and the 24-hour urinary excretion of 17-ketosteroids was 6.4 mg. After withdrawal of cortisone therapy for eight weeks, the blood pressure remained at 190 to 195 systolic and 95 to 110 diastolic. Urinary 17-ketosteroids had not risen and measured 2.9 mg. per twenty-four hours. ACTHAR-gel was then administered in a dosage of 40 units twice daily, but on the second day of administration she complained of severe cramping pains bilaterally in the posterior subcostal regions and was hospitalized for three days. ACTHAR-gel was discontinued after the second day and the

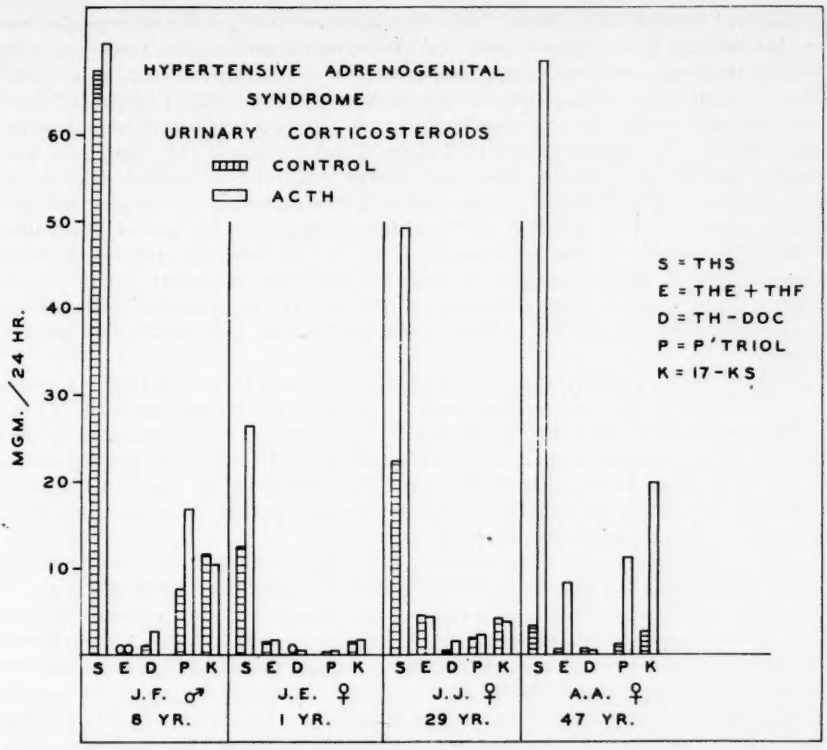

FIG. 1. Quantitative urinary corticosteroid pattern in 4 cases of hypertensive virilizing adrenal hyperplasia, before and during stimulation with ACTH.

pains disappeared. Analysis of the 24-hour urine specimen collected during the second day of ACTH stimulation showed tremendous rises in urinary corticosteroids (Fig. 1 and Table 1). Suppression therapy was reinstituted after discharge, and within four weeks the blood pressure had returned to a level of 140/100 where it remained.

METHODS FOR MEASUREMENT OF STEROIDS

Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels (10), and total urinary 17-hydroxycorticosteroids by the method of Glenn and Nelson (11). For the measurement of the individual urinary 17-hydroxycorticosteroids, the eluate obtained after chromatography on Florisil columns was divided into two equal portions and each portion applied on Whatman #2 paper. Chromatography was carried out using the Bush (12) system of solvents, benzene:methanol:water, 100:55:45. Reference steroids were chromatographed alongside the extracts. The paper strip corresponding to one of the two aliquots was then stained with blue tetrazolium (BTZ) and the parallel areas of the unstained strip corresponding to the spots obtained with blue tetrazolium were cut out and eluted by the wick technique of Bush, using ethanol (13). The ethanol eluate was evaporated to dryness and divided into equal portions;

one portion was used as a "blank" with ethanol-sulfuric acid and the other portion was treated with the Porter-Silber reagent (14). When the aliquots applied to the paper are kept in the range below 40 micrograms, recoveries of standards average 85 per cent. For the quantitation of tetrahydrodesoxycorticosterone (TH-DOC), a similar technique was followed but the solvent system of DeCourcy (isooctane:toluene:methanol:water, 25:75:80:20) was utilized for the separation of this compound (15). Standards were applied and the corresponding spots were located with BTZ on parallel strips in the described manner, and eluted. Quantitation was then performed by utilizing the reaction of Chen *et al.* (16). Accuracy in this method is limited by the lack of an adequate "blank." An eluate from the paper strip on which the standards were run was employed for estimation of "blank" readings. Urinary 17-ketosteroids were determined by a modification of the method of Callow, Callow and Emmens (17). Pregnanetriol measurements were performed by the method of Bongiovanni and Eberlein (18), modified for use with Florisil.

The values listed in Table 1 were corrected for the losses that occur in the process of paper chromatography, as computed from recoveries of the known standards. No correction was included for losses elsewhere in the procedure, in particular during the first step of Florisil chromatography. In a number of cases, THE and THF are reported as a combined value, for it appeared from the BTZ staining on the paper that accurate separation by eluting the individual spots would not be possible, perhaps owing to the presence of allotetrahydro derivatives.

Identification of the individual steroids, THE, THF and THS, was considered adequate according to the following criteria: mobility on paper in the Bush B-5 system to correspond with known standards, failure to absorb ultraviolet light, a positive reaction with blue tetrazolium, and the presence of a peaked spectral curve with phenylhydrazine-ethanol-sulfuric acid characteristic of those steroids possessing 17,21-dihydroxy-20-ketone side-chains. For the identification of tetrahydro-DOC, the criteria of mobility in the system of DeCourcy and the reaction with BTZ are less specific. Therefore enough material was isolated from the urine of Patient A.A. to permit identification by infrared spectroscopy.

RESULTS

Control values

The results of fractionation studies are listed in Table 1. In Figures 1 and 2, the results in the 4 patients with hypertension and 5 of the patients without hypertension are depicted graphically for purposes of comparison. It is apparent that the majority of patients with congenital adrenal hyperplasia demonstrate some ability to produce cortisol. This conclusion assumes that urinary tetrahydrocortisol and tetrahydrocortisone are derived only from endogenous cortisol. There is no evidence to the contrary. Only 1 of the 3 patients with hypertension failed to demonstrate this ability. Of the 6 infants with the severe salt-losing syndrome, 5 failed to excrete detectable amounts of THE or THF. All the remaining patients excreted amounts of tetrahydrocortisone consistent with normal amounts for their age and size. In all of the nonhypertensive patients, urinary 17-ketosteroids were elevated to diagnostic values. In most cases, this was also true for the urinary pregnanetriol. In contrast, the 3 patients with hypertension who demonstrated ability to form cortisol did not demon-

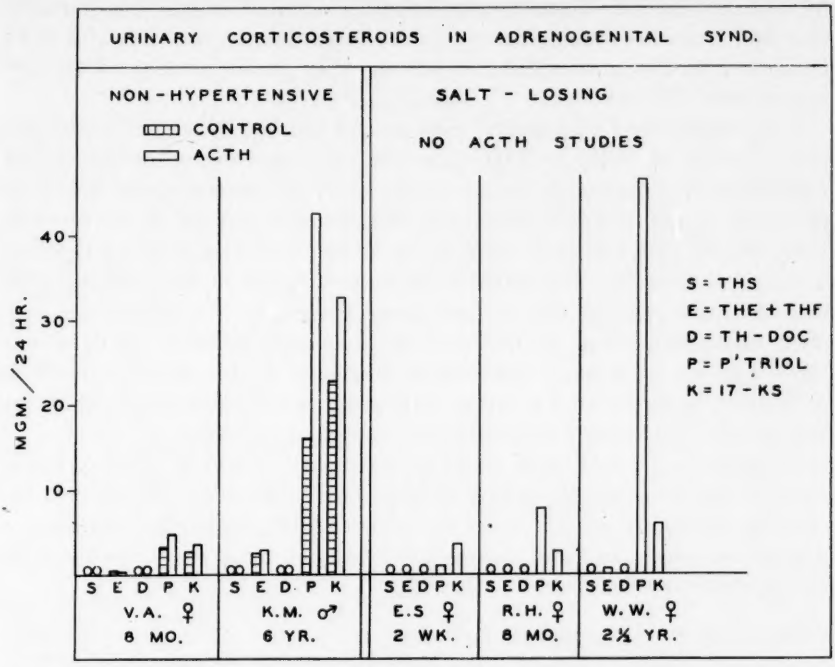

FIG. 2. Quantitative urinary corticosteroid pattern in 5 representative cases of non-hypertensive virilizing adrenal hyperplasia, 2 of the simple virilizing type (before and during ACTH stimulation) and 3 of the salt-losing type (untreated).

strate diagnostic elevations of 17-ketosteroids or pregnanetriol at the time of study. Certain qualifications must be pointed out. Patient A. A. had received small doses of cortisone for one month but had been without therapy for eight weeks at the time of this study. Because she had not been studied before treatment, we have no information concerning the initial levels of 17-ketosteroids. Patient J. J. had been treated with cortisone for six and a half years but all therapy had been withdrawn five months prior to this study. Before treatment, the 24-hour urinary excretion of 17-ketosteroids averaged 33 mg. Previous studies have been published concerning her pattern of ketosteroid excretion at that time (19). Pregnanetriol determinations on a preserved specimen of that original urine showed an excretion of 3.2 mg. per twenty-four hours—an equivocal result for diagnostic purposes.

ACTH studies

ACTHAR-gel was given intramuscularly in divided dosage for three days in an attempt to obtain maximal stimulation of adrenal production. Those patients critically ill with the salt-losing syndrome were not given

ACTH, for they were considered to be under maximal endogenous stimulation and previous studies have shown that administration of ACTH to patients with the adrenogenital syndrome may predispose toward further loss of salt (20, 21).

It is evident that stimulation with ACTH did not significantly increase the excretion of THE or THF in either the hypertensive or the simple virilizing forms. Although Patients A.A. and S.W. showed some degree of response, it was of small magnitude. The finding of THS in the urine of Patient S.W. in response to ACTH is interpreted as further proof that she was able to overcome her defect of 21-hydroxylation under great stimulation. It is also evident that in most cases 17-ketosteroids did not increase after administration of ACTH. It might be concluded from the data that these patients were under maximal endogenous ACTH stimulation. It is of interest, however, that in some of the cases, ACTH did cause significant increases in the urinary metabolites of precursor substances.

Therefore, it would seem that the enzymatic defect of hydroxylation which exists in each of these 3 forms of adrenal hyperplasia can not be overcome by additional ACTH, even though such stimulation may increase the amount of precursors and theoretically present more substrate material to the defective enzyme.

Internal check of degradation pathways

After completion of the periods of study, Patients J.J. and J.E. were treated with triamcinolone to inhibit adrenocortical secretion. In the quantities used, this drug does not produce significant levels of BTZ-reacting substances in the urine, and with totally suppressive doses, no reducing substances are evident on a paper chromatogram. A single dose of oral cortisol was then administered to each of these patients, and the urine was analyzed for the presence of excretory products. No THS was found, thus ruling out the remote possibility that THS in these persons might result from metabolic breakdown of cortisol.

DISCUSSION

The enzymatic defect

The biochemical theories for the explanation of the clinical syndrome of congenital adrenal hyperplasia have been thoroughly presented in the literature (22–24). Utilizing the theories of Hechter *et al.* (25, 26) and Hayano and Dorfman (27), Jailer *et al.* provided an explanation for the nonhypertensive forms of this syndrome (28). They suggested that a defect of the sequence of hydroxylation processes in the adrenal gland might result in defective synthesis of cortisol and corticosterone, thus resulting in the accumulation of precursor substances which are only partially hydroxylated

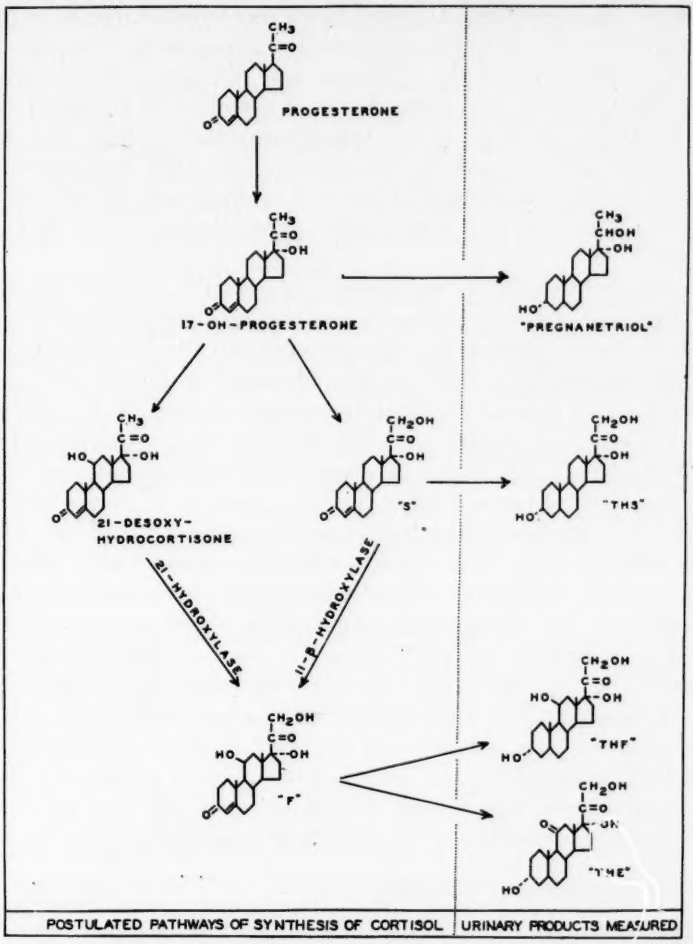

FIG. 3. Diagrammatic representation of the accepted theories of cortisol synthesis, with portrayal of the 4 major urinary metabolites measured in this study.

in the sequential enzymatic processes. Further studies by Jailer *et al.* (28) and Bongiovanni *et al.* (24) on the metabolism of suspected precursors seemed to confirm the theory of an enzymatic defect. *In vitro* studies of adrenal homogenates from patients with congenital adrenal hyperplasia (29) have added further convincing evidence of a defect, so that the present concept of the biogenesis of the syndrome has emerged as outlined in Figure 3. Deficiency of the enzyme labeled "21-hydroxylase" may be seen to interfere with the route to synthesis of cortisol, with resultant accumu-

lation of precursor products, the most familiar of which shown to be present is pregnanetriol. In 1955, Eberlein and Bongiovanni (4) discovered that the hypertensive form of the syndrome was different biochemically, but could be explained by the same theory of defective biogenesis if one assumed an absence of adrenal "11-beta-hydroxylase" rather than the accepted theory of deficiency of "21-hydroxylase." The hypertension was attributed to the accumulation of secreted 11-desoxycorticosterone (DOC) as a result of the same enzymatic defect in the synthetic pathway to corticosterone.

Data in the literature at the present time would indicate that the hypertensive form of this syndrome is due to a complete absence of adrenal "11-beta-hydroxylase" activity. Bongiovanni and Eberlein have published reports of 2 patients in whom no steroids could be found that possessed oxygen functions at position 11 on the steroid nucleus (4, 8). Dyrenfurth *et al.* have reported studies on a patient with the hypertensive form of congenital adrenal hyperplasia in whose urine small amounts of products were found that contained oxygen atoms at position 11 on the steroid nucleus (9). The studies carried out in our laboratory would indicate that cases of this defect may exist in attenuated forms not readily diagnosable as true cases of congenital adrenal hyperplasia. It is apparent from the data that only 1 of our patients exhibited the classic pattern of total absence of 11-hydroxylase activity. This was the boy who became virilized at an early age. The two older patients (sisters) with less complete defects, did not become virilized until puberty had become established. The presence of apparent true female sexual precocity in one of the sisters (J.J.) is a remarkable coincidence, but unfortunately there is no chemical or other evidence to support speculation concerning its origin. The occurrence is mentioned only to emphasize the absence of suspicion of early virilization.

Responses to withdrawal of therapy

At the time of study of Patient J.J. (Case III), she had been treated with cortisone for six and a half years. Initially, 17-ketosteroid excretion had been elevated, but after withdrawal of cortisone for five months and subsequent stimulation with ACTH, the total 17-ketosteroids failed to rise above low-normal levels. In the usual forms of congenital adrenal hyperplasia, this lack of response is most unusual. Several patients in this clinic with the simple virilizing form of congenital adrenal hyperplasia have recently been withdrawn from therapy after five years of daily suppressive doses of cortisone. One of them (D.B.) is represented in Table 1; the data show high excretions of 17-ketosteroids and pregnanetriol after an eighteen-month period of withdrawal. This boy's urinary 17-ketoster-

oids had risen to 28 mg. per twenty-four hours after two months without cortisone therapy. The usual pattern in these patients is a rebound of the urinary output of 17-ketosteroids to abnormally elevated levels within three to six months, even though the levels may not reach those present prior to therapy (30). The failure of hypertensive Patient J.J. to show this typical rebound suggests that the synthetic pathways of adrenal androgen synthesis may have been altered, for hirsutism progressed in this interval in spite of stabilized levels of urinary ketosteroids. This phenomenon of an altered pattern of urinary ketosteroid excretion has been reported previously in females with postpubertal hirsutism (6, 7).

In a case reported by Kappas *et al.* (6) an eleven-month follow-up showed that the ketosteroids still had not risen to the elevated pretreatment levels, but it was not stated whether the hirsutism was progressing. Both Patients A.A. and J.J. would perhaps fall in this category of "acquired" postpubertal virilism except for the fact that they were sisters, thus implicating genetic factors to account for the qualitative similarity of their patterns of urinary corticosteroid excretion. The quantitative differences in these patterns may have been due to the effects of the extreme differences in duration of suppressive therapy prior to this study.

Diagnosis

Of great interest is the observation that in an occasional case the usual laboratory criteria for the diagnosis of the adrenogenital syndrome may be misleading when applied to the hypertensive form of this disease. Urinary 17-ketosteroids, even under great stimulation with ACTH, may not rise to abnormal levels. Nevertheless, virilization may progress. This raises the possibility that the metabolic pathways of synthesis and degradation of adrenal androgens are altered in this syndrome in such a way that biologically active androgenic substances are present but cannot be detected in the neutral ketonic fraction of the urine that is assayed for 17-ketosteroids. The possibility that nonketonic substances are present in the urine of these patients in greater than normal amounts is presently under investigation in this laboratory. It is also apparent from our data that the measurement of pregnanetriol by the usual techniques would be diagnostically unreliable in some cases of the hypertensive syndrome. This is to be expected, for there is no evidence that a deficiency of "adrenal-21-hydroxylase" allows pregnanetriol to accumulate. In the hypertensive syndrome, the major precursor metabolite that accumulates in the urine is tetrahydro-S. At the present time, the only simple method of separating this substance from the usual breakdown products of cortisol is by a paper chromatographic procedure. Utilization of this method may be necessary before dis-

carding the possibility of congenital adrenal hyperplasia when faced with the diagnostic considerations presented in such cases as Patients A.A. and J.E.

Data obtained from the literature and in this laboratory would indicate that tetrahydro-S is present in very small amounts in the urine of persons without congenital adrenal hyperplasia. It has previously been found in cases of adrenal carcinoma in amounts up to 12 milligrams per twenty-four hours (31, 32). Touchstone *et al.* reported that this substance is present in pooled collections of urine from normal subjects in a quantity that would indicate an average excretion of 20 micrograms per day (33). One hypertensive male was found to excrete 70 micrograms per day; none was found in the urine of 2 hypertensive females. In none of these patients was it present in the quantities found in adrenal hyperplasia and adrenal carcinoma. The techniques utilized in the present study did not permit discovery of amounts less than 100 micrograms per day. It was felt that in detection of cases of this syndrome, more sensitive methods were not necessary since the greater fraction of alpha-ketolic urinary corticosteroids would be present as tetrahydro-S.

In an attempt to ascertain the possible incidence of this disease in a large population, 88 subjects were taken at random from the 350 persons attending the hypertensive clinic at this hospital. In addition, 25 hirsute females with moderate to severe elevations of blood pressure were selected from the gynecology clinics of this hospital and two neighboring hospitals. None of these 113 subjects was found to excrete significant amounts of tetrahydro-S, indicating the relatively rare occurrence of this syndrome as manifested in Patient A.A. In spite of the negative results of this survey, it would seem advisable to look for evidence of this syndrome in all hirsute, hypertensive persons.

Variation in the degree of enzymatic defect

It is apparent that in the majority of cases of the adrenogenital syndrome, the enzymatic defect is incomplete. Most nonhypertensive cases studied previously have not demonstrated total defects of cortisol synthesis (8, 9, 34, 35), but this fact has not been evident in the hypertensive form. In the nonhypertensive forms, Eberlein and Bongiovanni pointed out that there seems to be a correlation between the degree of defect and the degree of salt-losing tendency (8). The data from our group of patients would support that hypothesis in most cases. However, the methods of analysis of urinary corticosteroids depend upon enzymatic hydrolysis with β-glucuronidase and subsequent extraction into an organic solvent. It is now well established that in the neonatal period, conjugation and excretion of steroids as glucuronides is not the chief excretory pathway (36).

Thus, the finding of low or zero excretion of corticosteroids in the urine of salt-losers, most of whom are neonatal subjects, may not be a true indication of their production of cortisol. Blizzard *et al.* (37) recently demonstrated that salt-losers (aged $1\frac{3}{4}$ to 9 years) appear incapable of responding to the stress of salt-deprivation by an increase in the production of aldosterone. Since the production of aldosterone and cortisol presumably is dependent upon the same enzymatic process for hydroxylation at position 21, such data would be in agreement with the concept that the necessary enzymatic processes are most defective in those patients demonstrating the most serious symptoms.

In the hypertensive form, such a dichotomy is not evident. Clinically, there does not appear to be any correlation between the degree of hypertension and the degree of enzymatic defect, for those with the least defect may have severe hypertension. The variable factor has been the age at onset of virilization and hypertension. The phenomenon of the two sisters (J.J. and A.A.) linking the hypertensive form of congenital adrenal hyperplasia with a postpubertal form of virilism and hypertension, lends further evidence to the concept of a spectrum of related abnormalities in adrenal enzymes accounting for variable expressions of virilism in females. In 1954, Jones and Jones (38) suggested a classification for hirsute, oligomenorrheic females dependent upon the degrees of postulated adrenal defects. Kappas *et al.* (6) suggested that a continuum would be found between the extreme of congenital adrenal hyperplasia and the normal state. Additional evidence of a spectrum of abnormalities has been offered by Bush (7), in a thorough and detailed study of twin sisters. These authors found evidence of androgenic abnormalities only, and in the paper of Kappas the assumption was made that no defect existed in corticosteroid synthesis since clinical signs of hypoadrenocorticism did not exist and the excretion of urinary corticosteroids was not decreased. It is apparent from our study that one must also seek for evidence of precursor substances, since most patients with congenital adrenal hyperplasia do excrete normal amounts of cortisol end-products.

In comparing the hypertensive, salt-losing and simple virilizing forms of congenital adrenal hyperplasia, it has been well established that siblings may present similar forms of the disease, and that these forms are not mixed within the same family tree. These facts have been interpreted to demonstrate the genetic mode of inheritance of this syndrome (39). The finding that in some of these patients in all categories the enzymatic defects are not absolute, would suggest that there are varying degrees of defective enzymatic function within the three sub-groups of congenital adrenal hyperplasia. For our 4 cases of the hypertensive type, one could postulate variant genes at the same locus. Thus, the defect would express

itself consistently within family groups, but to varying degrees in the population as a whole. The numbers of known cases in this category are too small for definite conclusions, but the variability manifested by the 4 hypertensive cases presented here would suggest that this may be the case.

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VASOPRESSIN AND OXYTOCIN IN THE POSTERIOR LOBE OF THE PITUITARY OF MAN*

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ABSTRACT

The posterior pituitary lobes from 9 patients, 6 female and 3 male, who died from a variety of illnesses were assayed individually for vasopressin (vasopressor and antidiuretic responses) and oxytocin (milk-ejection activity). There was a remarkable variation in the vasopressin/oxytocin ratio (0.2–2.4) and this suggests that the hormones are not present in a fixed proportion bound to a protein in the glands in man. Age, sex, stress as assessed by the lipid pattern in the adrenal cortex, the disease state, and steroid therapy had no obvious influence on the yield of vasopressin and oxytocin in the small series of cases studied.

THE purpose of this report is to increase the small amount of information available with respect to the quantities of neurohypophysial hormones in the posterior lobe of the human pituitary gland. The possibility that the amounts of stored hormone could be correlated with age or with stress preceding death was investigated. No rough correlation between stress and hormone content sufficient to justify a more extensive investigation was observed.

METHODS AND MATERIALS

Source of material. Nine posterior hypophysial lobes were collected two and a half to six hours after death from 3 men and 6 women who died from various systemic diseases (Fig. 1).

Pre-assay treatment of material. The posterior lobe was dissected off immediately after collection of the gland and placed in a closed container at −18° C. The duration of storage of frozen individual glands varied from a few weeks to a few months. The lobes were bisected in most cases before being dried in a frozen state for sixty-five to seventy

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hours at a temperature of −20° to −25° C. and a pressure of about 0.01 mm.Hg. Under sterile conditions the lobes were then cut into very small pieces and immersed in acetone (acetonum, B.P.). They were shaken at intervals in several changes of acetone over a period of several hours at room temperature, transferred to an ampule and dried in a desiccator over P_2O_5 in an incubator at 37° C. *in vacuo.*

The dried tissues were triturated and suspended in an aqueous solution (pH 3.0) containing 0.3 per cent acetic acid and 0.5 per cent chlorobutanol. Extraction was carried

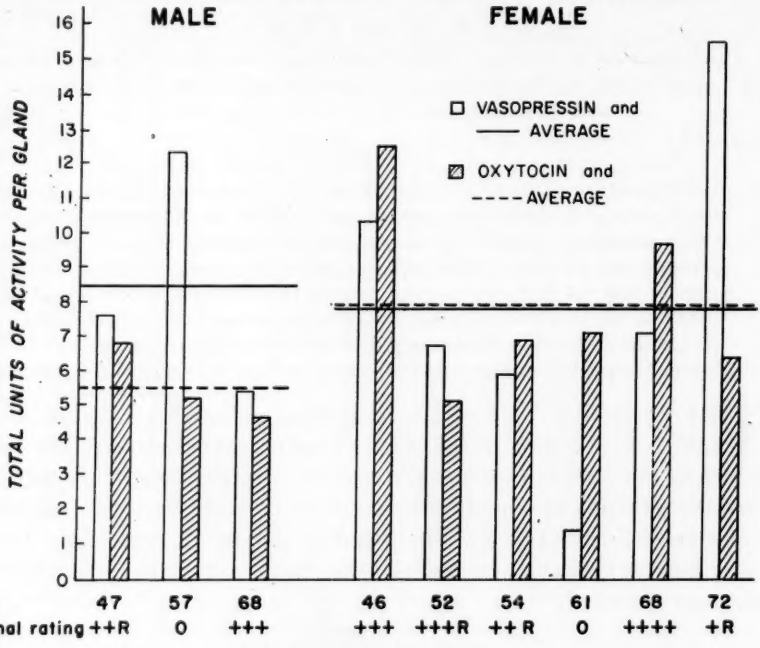

FIG. 1. Amounts of vasopressin and oxytocin in human posterior hypophysial lobes arranged according to sex, age and adrenal rating.

out in a boiling water bath for ten minutes. Each milliliter of extraction fluid represented 1 milligram of dried tissue. After centrifugation for removal of tissue, the clear supernatant fluid was transferred to ampules which were sealed, immersed in boiling water for ten minutes, and then stored at 2° C. All activities are stated in terms of the International Standard.

Assessment of adrenal changes and degree of stress. The degrees of stress were graded according to the method of Currie and Symington (1, 2) which is based on the adrenal lipid pattern and on the clinical history. The extent of lipid depletion of the adrenal cortex reflects to some degree the severity of the stress to which the patient has been subjected before death, and five grades of depletion are recognized 0, +, ++, +++ and ++++. Grade 0 signifies that the adrenal shows no lipid depletion; it is usually found in cases of sudden death or of chronic illness. At the other extreme, Grades +++ to ++++ indicate that the adrenal contains little or no demonstrable lipid and the

patient in most instances has suffered from a severe stressing illness such as an extensive burn or a severe infection, or has undergone a major operation a few days before death. Grades + and ++ indicate an adrenal which is structurally between the two extremes. Sometimes the lipid in a partially depleted gland shows reversion of the normal pattern and is present in the deeper part of the zona fasciculata while the superficial part of the zone is depleted of lipid (designated R in Figure 1). These adrenals are apparently becoming restocked with lipid after a period of hypersecretion of adrenocortical hormones and are returning to normal function and structure. It is emphasized that the gradings are mainly determined by the structural changes in the adrenal cortex and do not necessarily represent fine degrees of adrenocortical functional activity or of physical stress.

Methods of assay. Vasopressor, antidiuretic and oxytocic activities were determined quantitatively according to the methods used by van Dyke, Adamsons and Engel (3). The anesthetized rat was used for vasopressor assays by a technique modified from that of Landgrebe, Macaulay and Waring (4). Assays of antidiuretic activity were performed in hydrated unanesthetized dogs. Oxytocin was determined by changes in the milk-ejection pressure of a breast of the anesthetized lactating rabbit after a suitable correction for the amount of vasopressin present. The diluted extracts were injected intravenously in all the assays.

The estimates of vasopressin (Fig. 1) are averages of vasopressor and antidiuretic activities which in human pituitaries represent arginine vasopressin. (Generally, the estimates of vasopressin were slightly higher by antidiuretic assay than by vasopressor assay; the differences could not be regarded as significant.) The milk-ejection activity of arginine vasopressin was assumed to be 17 per cent of that of oxytocin for the purpose of correcting for the vasopressin in estimating the units of oxytocin in extracts.

RESULTS AND DISCUSSION

All the results, which are shown graphically in Figure 1, are expressed in terms of the total units of vasopressin and oxytocin. The average weight of the dried, acetone-extracted posterior lobes was 14.0 mg. and there was no significant difference between the group of males (15.6 mg.) and the group of females (13.3 mg.). In the combined groups, total oxytocin averaged 7.14 units with a standard deviation of ± 2.51 units. It showed less variation (coefficient of variation, 0.35) than did vasopressin (8.63 ± 4.61 units; coefficient of variation, 0.53). The total amount of neither hormone could be correlated with age, but the youngest patient studied was 46 years old. Essential hypertension or adrenal steroid therapy a few days before death was without apparent effect on the hormone content of the glands. There was no correlation between the yield of vasopressin and oxytocin and the lipid pattern of the adrenal cortex (Fig. 1). The adrenal lipid content does not necessarily reflect the corticosteroid level in the blood, and the level of circulating corticosteroids was not estimated. The assessment of the degree of stress, using the adrenal lipid pattern as the index, is admittedly crude. The rate of synthesis of hormone in the human posterior pituitary gland cannot be estimated, and the amount stored is a balance between the amount synthesized and the amount released. If

the rate of synthesis were unable to keep up with the rate of release this would result in low storage. On the other hand, if the rate of synthesis were very rapid this might permit release of large amounts of hormone without depletion of the amount stored in the gland.

The ratio of vasopressin to oxytocin (V/O) has been found to vary among different mammals, and ranges from 1.1 to 3.3 in normal camels, dogs, hogs, oxen, rats and monkeys. In a previous study of 3 human posterior lobes, the V/O was about 1.1 (van Dyke *et al.* (5)). In the present series of 9 human glands, the V/O ranged from 0.2 to 2.4, with an average of 1.3. These findings suggest that in the human posterior lobe, vasopressin and oxytocin are not bound in fixed proportions to a protein such as can be extracted from the posterior lobes of oxen (van Dyke *et al.* (6)). It is possible, however, that the inconsistency of the ratio between vasopressin and oxytocin may represent an agonal response and not measure the state of affairs under normal physiologic conditions.

There are few satisfactory reports concerning the total quantities of the hormones in the human posterior hypophysial lobe. Jores and Zschimmer (7), estimating only oxytocin, found remarkably low total quantities such as an average of 2.3 units in 33 whole pituitaries or 1.35 units in 38 posterior lobes. Simon and Nagy (8) reported averages of 8.7 units of vasopressin and 9.6 units of oxytocin in 32 specimens from patients 18 to 79 years old. According to Heller and Zaimis (9), who used specimens from 13 patients aged 14 to 64 years, the average amount of antidiuretic hormone (vasopressin) was 14.6 units and that of oxytocin was 13.9 units.

From the work just mentioned and from the findings of the present report it may be concluded that no difference in the quantity of either hormone can be related to sex. The ratio of vasopressin to oxytocin varies widely for reasons which are unknown; the *average* ratio is about 1.0 to 1.3.

The functional significance of the yield of hormones in the posterior pituitary lobe is not known and this study does not help to decide what relative importance should be assigned to production, storage and release of the hormones on the basis of determinations made *post mortem*.

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THE NATURAL HISTORY OF THE DELTA CELL OF THE HUMAN ADENOHYPOPHYSIS: IN CHILDHOOD, ADULTHOOD, AND PREGNANCY*

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ABSTRACT

Using the iron-PAS stain, counts were made of the cells in the pituitaries of 69 children and 44 pregnant or postpartum women. The results were analyzed statistically. No delta cells were found before the age of 10, after which the percentage of delta cells gradually increased. Also, no delta cells were found after the first trimester of pregnancy. These observations suggest that delta cells produce and store gonadotropin. A further analysis of data obtained on a group of 71 nonendocrine adult patients showed a greater percentage of delta cells in men past the age of 60 than in women of corresponding age. On the other hand, the pituitaries of these women contained a higher percentage of gamma cells. These gamma cells probably also secrete gonadotropin, but store little of the hormone.

INTRODUCTION

TWO types of basophils, beta and delta, can be seen in human pituitaries stained with the iron-PAS technique. These cells have been described in previous publications (1). In these studies, the staining properties, size, shape and granulation of the delta cell, as well as the random distribution throughout the adenohypophysis, seemed to establish a relationship between it and the gonadotrope observed in the pituitaries of various animal species *e.g.*, rats (2, 3), cattle (4) and frogs (5). The percentage of delta cells appeared to be related to the gonadotropic activity of the pituitary at various stages of life: no delta cells were seen in the pituitary during childhood or pregnancy, at which time there is little or no secretion of gonadotropins by the pituitary (6–8). The greatest proportion of delta cells was found in the pituitaries of adults who had died suddenly; the incidence decreased sharply with increasing duration of the final illness (1).

In previous studies of the distribution of the cell types in the adenohypophysis, the cells were divided into three categories: acidophils, basophils and chromophobes. Neither the delta cell nor the gamma cell were recog-

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nized as separate entities (9–14). The present work was undertaken as a systematic study of the effects of age, sex and pregnancy on the proportion of delta cells, as well as of gamma cells, alpha cells (classic acidophils), beta cells (classic basophils) and chromophobes.

METHODS

The pituitaries of 69 children and 44 pregnant or postpartum women were obtained from various hospitals in the Toronto and Montreal areas.[1] The techniques of fixation, staining and counting of cells have been described in previous papers (1). Cell counts performed previously on the pituitaries of 71 adult nonendocrine patients (1) were included in the statistical treatment.

The cases were divided into the following groups for purposes of statistical analysis:[2]

A. Children— 1) infants (0–5 years).
2) pre-pubertal (6–10 years).
3) pubertal (11–15 years).
4) post-pubertal (16–20 years).

B. Adults— 1) young (21–40 years); the females were all presumed to be pre menopausal.
2) middle-aged (41–60 years); some females were premenopausal some were in the menopause, and some were postmenopausal.
3) elderly (more than 60 years).

(Males and females were considered separately, as well as in combination.)

C. Pregnancy— 1) first trimester (0–3 months).
2) second trimester (4–6 months).
3) third trimester (7–9 months).
4) post partum (2 months).

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[1] The authors wish to acknowledge the help of Dr. N. W. Roome, of the Committee for the Study of Maternal Deaths, Department of Health of Ontario, in locating cases of maternal deaths in the Toronto area during the period 1953–1957. We also thank the following pathologists for their co-operation in providing us with material and case histories: Dr. H. T. Van Patter of St. Michael's Hospital, Toronto; Dr. H. G. Pritzker, of the New Mt. Sinai Hospital, Toronto; Dr. S. F. Penny, of the Toronto East General Hospital; Dr. L. S. Mautner, of St. Joseph's Hospital, Toronto; Dr. F. Jaffe, of the Toronto Western Hospital; Dr. D. L. Donohue, of the Hospital for Sick Children, Toronto; Dr. R. Ritchie, of the Northwestern General Hospital, Toronto, and the Women's College Hospital, Toronto; Dr. R. Clark, of the Oshawa General Hospital; and Dr. H. Root, of the Montreal General Hospital.

[2] Calculations were carried out by use of the IBM-650 digital computer in the Computation Centre of the Department of Physics, University of Toronto. We wish to thank Dr. W. H. Watson, Professor and Head of the Department of Physics, and Director of the Computation Centre, for his encouragement and permission to use the computer; and the Computation Centre staff, and Mr. G. L. Lunchick, for their co-operation. Also we wish to thank Dr. G. C. Gotlieb, Chief Computer, and Dr. L. C. Lax, and Dr. B. H. Worsley, for their help with the programming.

The mean with its standard error was found for each group, and *t* tests were performed regarding the significance of differences of the means between all the groups in the children's categories and all the groups in the adults' categories. The mean for each of the age-groups in the children's categories was also compared with the mean for the adults combined for all ages. The means for all 3 pregnancy groups and for the postpartum group were compared singly and in combination with each other, as well as with the mean for the adult nonpregnant females combined for all ages. Linear regressions were calculated for the percentage of alpha cells and chromophobes versus age in the age groups from 0–20, and for the percentage of gamma cells versus age throughout the life span (exclusive of pregnancy).

RESULTS

The effect of age, sex and pregnancy on each cell type will be considered separately. The percentages of the various cells in the pituitaries of patients in the different groups, and their standard errors, are summarized graphically in Figure 1. In Table 1–5 are listed the percentages of the total cell population represented by delta cells, gamma cells, alpha cells, beta cells and chromophobes, respectively, as well as the statistical significance of the differences between groups. Cell counts for the individual cases in the children's and pregnancy groups are not reported because of limitation of space.[3] The data for the individual adults have been published previously (1).

Delta cells (Table 1, Figs. 1 and 2)

Cell counts were performed on the pituitaries of 28 boys and 15 girls under 10 years of age. No delta cells were seen in any of the pituitaries in spite of the fact that 13 of these children had died suddenly. It was observed previously that in adults the percentage of delta cells was highest in cases of sudden death (1). Figure 2 illustrates the relationship between individual values for the percentage of delta cells in the pituitary, and the age and sex of the patients and the duration of their final illnesses. A few delta cells appear in some pituitaries at age 10, and their incidence gradually increases throughout puberty. The percentage of delta cells increases significantly in the 11–15 year group, but does not increase further in the 16–20 year group. The proportion of delta cells in the subjects ranging in age from 11 to 20 years is significantly less than in adults past 20 years of age. Sex bears no relationship to the cell counts in children's glands. When cell counts in glands from adults whose fatal illnesses varied in duration are grouped according to the age of subject, no significant influence of age is detectable. The menopause does not seem to influence the proportion of delta cells. However, among adults, the percentage of delta cells in the

[3] Detailed information on these cases is available on request.

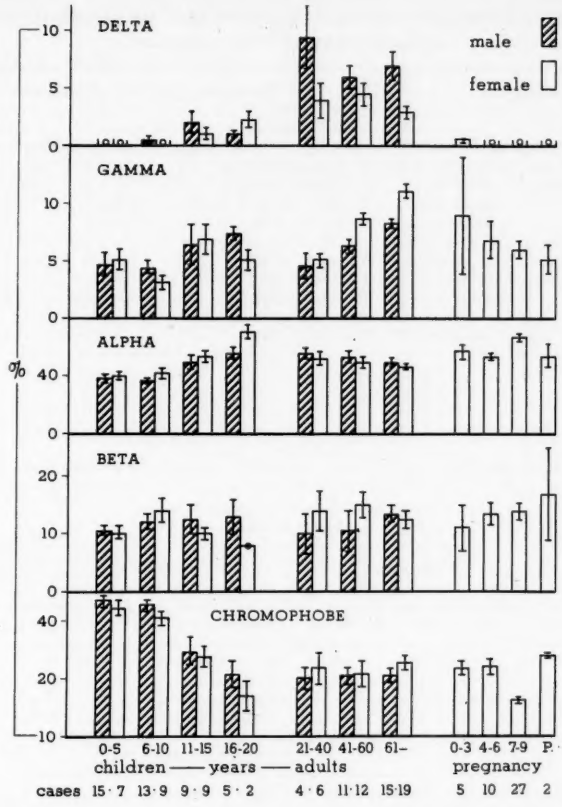

FIG. 1. Proportions of pituitary cell types in relation to age, sex and pregnancy. The short crossbars joined by a vertical line indicate the standard error of the mean.

male is significantly greater than in the female; this difference is particularly marked in the age group over 60.

In pregnancy, delta cells were found only in 2 patients who died in the first trimester. In several cases the pregnancy was terminated prematurely, but death did not occur until several days later; in none of these cases were delta cells found. Delta cells were also absent from the pituitaries of 2 patients who died two months after delivery at term; both of these women were severely ill during the postpartum period.

An unusual type of cell was seen in a case of chorionepithelioma in a 10-year-old boy who had matured sexually, and in another case of chorionepithelioma in an adult. These cells were polyhedral in shape, like beta cells, but with homogeneous purple-straining cytoplasm. Their function is obscure. They were classified as a sub-type of delta cell ("diffuse").

TABLE 1. EFFECT OF AGE, SEX AND PREGNANCY ON THE PROPORTION OF DELTA CELLS IN THE PITUITARY

		Delta cells (% of total cell population)		
		In males	In females	Combined M & F
Children	Age (yrs.) 0–5*	0 [15]†	0 [7]	0 [22]
	6–10	0.4±0.3** [13]	0 [9]	0.9±0.09 [22]
	11–15	2.0±0.9 [9]	1.1 ±0.4 [9]	1.6±0.5 [18]
	16–20	0.9±0.3 [5]	2.1 ±0.7 [2]	1.2±0.3 [7]
Adults	Age (yrs.) 21–40	8.8±2.8 [4]	3.9 ±1.6 [6]	5.9±1.6 [10]
	41–60	6.6±1.0 [11]	4.5 ±1.1 [12]	5.5±0.8 [23]
	over 60	6.9±1.2 [15]	3.0 ±0.6 [19]	4.7±0.7 [34]
	All adults combined	7.0±0.8 [30]	3.6 ±0.5 [37]	5.2±0.5 [67]
Pregnancy	Mos. 0–3		0.28±0.2 [5]	
	4–6		0 [10]	
	7–9		0 [27]	
	Post partum		0 [2]	
	Combined 0–6		0.1 ±0.2 [15]	

Statistical analysis: P values less than 0.05

A—Effect of age

	Male		Female		Combined M & F	
	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P
Children	0–5 11–15	.01	0–5 11–15	.05	0–5 11–15	.001
	0–5 16–20	.001	0–5 16–20	.001	0–5 16–20	.001
	6–10 11–15	.05	6–10 11–15	.02	6–10 11–15	.01
			6–10 16–20	.001	6–10 16–20	.001
Adults			No significant differences			
Children *vs.* adults	0–5 >20	.001	0–5 >20	.01	0–5 >20	.001
	6–10 >20	.001	6–10 >20	.01	6–10 >20	.001
	11–15 >20	.01	11–15 >20	.05	11–15 >20	.001
	16–20 >20	.01			16–20 >20	.02

B—Effect of sex

	Male *vs.* female	P
Children	No significant differences	
Adults	M >60 *vs.* F >60	.01
	M >20 *vs.* F >20	.001

C—Effect of pregnancy

	P
Pregnancy 0–3 mos. *vs.* nonpregnant females >20	.05
Pregnancy 4–6 mos. *vs.* nonpregnant females	.01
Pregnancy 7–9 mos. *vs.* nonpregnant females	.001
Pregnancy 7–9 mos. *vs.* pregnancy 0–3 mos.	.01
Pregnancy 7–9 mos. *vs.* pregnancy 0–6 mos.	.001

* Includes all children from birth until age 6.
** Standard Error.
† Number of cases.

TABLE 2. EFFECT OF AGE, SEX AND PREGNANCY ON THE PROPORTION OF GAMMA CELLS IN THE PITUITARY

		Gamma cells (% of total cell population)		
		In males	In females	Combined M & F
Children	Age (yrs.) 0– 5*	4.9±1.4** [15]†	5.2±1.1 [7]	5.0±1.0 [22]
	6–10	4.4±0.7 [13]	3.0±0.7 [9]	3.9±0.6 [22]
	11–15	6.3±1.8 [9]	6.9±1.6 [9]	6.6±1.2 [18]
	16–20	7.2±0.5 [5]	5.2±1.1 [2]	6.6±0.6 [7]
Adults	Age (yrs.) 21–40	4.7±1.2 [4]	5.3±0.9 [6]	5.1±0.7 [10]
	41–60	6.8±0.9 [11]	8.8±0.7 [12]	7.8±0.6 [23]
	over 60	7.9±0.8 [15]	11.4±0.8 [19]	9.9±0.6 [34]
	All adults combined	7.1±0.6 [30]	9.6±0.6 [37]	8.5±0.4 [67]
Pregnancy	Mos. 0– 3		9.0±5.0 [5]	
	4– 6		6.7±2.0 [10]	
	7– 9		6.1±1.0 [27]	
	Post partum		5.1±1.3 [2]	
	Combined 0– 6		7.4±2.1 [15]	

Statistical analysis: P values less than 0.05

A—Effect of age

	Male		Female		Combined M & F	
	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P
Children	No significant differences		6–10 11–15	.05	6–10 11–15	.05
					6–10 16–20	.02
Adults	No significant differences		21–40 41–60	.01	21–40 41–60	.02
			21–40 >60	.001	21–40 >60	.001
			41–60 >60	.05	41–60 >60	.05
Children *vs.* adults	6–10 >20	.02	0– 5 >20	.01	0– 5 >20	.001
			6–10 >20	.001	6–10 >20	.001

B—Effect of sex

	Male *vs.* female	P
Children	No significant differences	
Adults	M >60 F >60	.01
	M >20 F >20	.01

C—Effect of pregnancy

	P
Pregnancy 7–9 mos. *vs.* nonpregnant females >20	.01

* Includes all children from birth until age 6.
** Standard Error.
† Number of cases.

Gamma cells (Table 2, Fig. 1)

The percentage of gamma cells is low in children of both sexes. In males, although the differences between individual age groups are small, the proportion of gamma cells in men is significantly greater than in boys. In

TABLE 3. EFFECT OF AGE, SEX AND PREGNANCY ON THE PROPORTION OF ALPHA CELLS IN THE PITUITARY

		Alpha cells (% of total cell population)		
		In males	In females	Combined M & F
Children	Age (yrs.) 0– 5*	37.9±2.6** [15]†	40.5±2.8 [7]	38.7±1.9 [22]
	6–10	37.7±1.7 [13]	42.4±3.8 [9]	39.6±1.9 [22]
	11–15	49.9±4.5 [9]	54.3±4.0 [9]	52.1±2.9 [18]
	16–20	56.5±4.8 [5]	70.6±4.4 [2]	60.5±4.3 [7]
Adults	Age (yrs.) 21–40	56.0±3.7 [4]	52.8±5.1 [6]	54.0±3.3 [10]
	41–60	54.3±4.0 [11]	49.6±4.3 [12]	51.8±2.9 [23]
	over 60	50.0±3.1 [15]	47.1±2.9 [19]	48.4±2.1 [34]
	All adults combined	52.4±2.2 [30]	48.4±2.2 [37]	50.4±1.5 [67]
Pregnancy	Mos. 0– 3		57.3±5.0 [5]	
	4– 6		54.7±3.0 [10]	
	7– 9		67.0±2.8 [27]	
	Post partum		53.9±7.6 [2]	
	Combined 0– 6		55.6±2.5 [15]	

Statistical analysis: P values less than 0.05

A—Effect of age

	Male		Female		Combined M & F	
	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P
Children	0– 5 11–15	.02	0– 5 11–15	.02	0– 5 11–15	.001
	0– 5 16–20	.01	0– 5 16–20	.001	0– 5 16–20	.001
	6–10 11–15	.01	6–10 11–15	.05	6–10 11–15	.001
	6–10 16–20	.001	6–10 16–20	.01	6–10 16–20	.001
Adults			No significant differences			
Children *vs.* adults	0– 5 >20	.001			0– 5 >20	.001
	6–10 >20	.001			6–10 >20	.001
			16–20 >20	.05	16–20 >20	.05

B—Effect of sex		**C**—Effect of pregnancy	
	Male *vs.* female		P
Children	No significant differences	Pregnancy 7–9 mos. *vs.* nonpregnant females >20	.001
Adults	No significant differences	Pregnancy 7–9 mos. *vs.* pregnancy 0–6 mos.	.01

* Includes all children from birth until age 6.
** Standard Error.
† Number of cases.

females, there is a gradual rise in the percentage of gamma cells throughout life, resulting in significantly more gamma cells in the pituitaries of women compared to those of young girls. There is a greater proportion of gamma cells in the glands of adult females than in those of adult males; the difference is particularly notable in the age group over 60. The equation for the regression of the percentage of gamma cells versus age (sexes

TABLE 4. EFFECT OF AGE, SEX AND PREGNANCY ON THE PROPORTION OF BETA CELLS IN THE PITUITARY

		Beta cells (% of total cell population)		
		In males	In females	Combined M & F
Children	Age (yrs.) 0– 5*	10.4±1.7** [15]†	10.1±1.6 [7]	10.3±1.2 [22]
	6–10	11.8±1.7 [13]	14.0+2.1 [9]	12.7±1.3 [22]
	11–15	12.7±2.4 [9]	10.0±1.2 [9]	11.4±1.4 [18]
	16–20	13.1±2.9 [5]	8.1±0.1 [2]	11.7±2.2 [7]
Adults	Age (yrs.) 21–40	10.2±3.4 [4]	13.8±3.6 [6]	12.4±2.5 [10]
	41–60	10.7±1.8 [11]	15.0±2.3 [12]	13.0±1.5 [23]
	over 60	13.6±1.7 [15]	12.7±1.4 [19]	13.1±1.1 [34]
	All adults combined	12.1±1.2 [30]	13.6±1.2 [37]	13.0±0.8 [67]
Pregnancy	Mos. 0– 3		11.2±3.8 [5]	
	4– 6		14.2±2.0 [10]	
	7– 9		14.0±1.6 [27]	
	Post partum		17.0±8.0 [2]	
	Combined 0– 6		13.3±1.8 [15]	

Statistical analysis: P values less than 0.05.
No significant differences between any groups.

* Includes all children from birth until age 6.
** Standard Error.
† Number of cases.

combined) throughout the life span is:

$$\% \text{ gamma} = 5.27 + 0.059 \text{ (age)}.$$

The regression coefficient is 0.059, and this slope is significantly different from zero (P = .001). The correlation coefficient is .414.

In the last trimester of pregnancy, gamma cells are reduced significantly.

Alpha cells[4] (Table 3, Fig. 1)

There are relatively few alpha cells until puberty, at which time their proportion increases significantly to reach the adult level. In the 2 girls in the 16–20 age group there were relatively more alpha cells than in the adult females. The percentage of alpha cells does not vary appreciably throughout adult life, and no sex differences are apparent at any age. The

[4] Romeis (15) has suggested that there are two types of acidophils in the human adenohypophysis—the alpha cell, which is carminophilic, and the epsilon cell, which is distinguished by its failure to stain with azocarmine. The epsilon cell takes up the orange-G counterstain used in Heidenhain's Azan technique, which Romeis employed. The addition of a carmine stain to the iron-PAS technique would create confusion between red alpha cells and red beta cells. In our study all of the "orange-G positive" acidophils were designated as alpha cells, although it is likely that some of them correspond to the epsilon cells described by Romeis.

TABLE 5. EFFECT OF AGE, SEX AND PREGNANCY ON THE PROPORTION OF CHROMOPHOBES IN THE PITUITARY

		Chromophobes (% of total cell population)		
		In males	In females	Combined M & F
Children	Age (yrs.) 0– 5*	47.0±2.6** [15]†	44.0±2.8 [7]	46.0±2.0 [22]
	6–10	45.3±2.2 [13]	40.3±2.6 [9]	43.2±1.7 [22]
	11–15	28.8±4.4 [9]	27.5±3.9 [9]	28.1±2.9 [18]
	16–20	21.2±4.7 [5]	14.0±5.1 [2]	19.2±3.7 [7]
Adults	Age (yrs.) 21–40	20.1±4.1 [4]	23.8±5.7 [6]	22.3±3.7 [10]
	41–60	21.2±3.0 [11]	21.7±4.4 [12]	21.5±2.6 [23]
	over 60	21.1±2.5 [15]	25.3±2.0 [19]	22.5±1.6 [34]
	All adults combined	21.0±1.7 [30]	23.9±1.9 [37]	22.6±1.3 [67]
Pregnancy	Mos. 0– 3		23.5±2.5 [5]	
	4– 6		24.1±2.7 [10]	
	7– 9		12.9±2.1 [27]	
	Post partum		28.0±0.2 [2]	
	Combined 0– 6		23.9±1.9 [15]	

Statistical analysis: P values less than 0.05

A—Effect of age

	Male		Female		Combined M & F	
	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P	Age groups compared (yrs.)	P
Children	0– 5 11–15	.001	0– 5 11–15	.01	0– 5 11–15	.001
	0– 5 16–20	.001	0– 5 16–20	.001	0– 5 16–20	.001
	6–10 11–15	.01	6–10 11–15	.02	6–10 11–15	.001
	6–10 16–20	.001	6–10 16–20	.01	6–10 16–20	.001
Adults			No significant differences			
Children *vs.* adults	0– 5 >20	.001	0– 5 >20	.001	0– 5 >20	.001
	6–10 >20	.001	6–10 >20	.001	6–10 >20	.001
	11–15 >20	.05				

B—Effect of sex

	Male *vs.* female
Children	No significant differences
Adults	No significant differences

C—Effect of pregnancy

	P
Pregnancy 7–9 mos. *vs.* nonpregnant females >20	.001
Pregnancy 7–9 mos. *vs.* pregnancy 0–3 mos.	.05
Pregnancy 7–9 mos. *vs.* pregnancy 4–6 mos.	.01
Pregnancy 7–9 mos. *vs.* pregnancy 0–6 mos.	.001

* Includes all children from birth until age 6.
** Standard Error.
† Number of cases.

equation for the regression of the percentage of alpha cells versus age (sexes combined) between the limits of 0 and 20 years is:

$$\% \text{ alpha} = 33.83 + 1.305 \text{ (age)}.$$

The regression coefficient is 1.305 and this slope is significantly different from zero (P = .001). The correlation coefficient is .567.

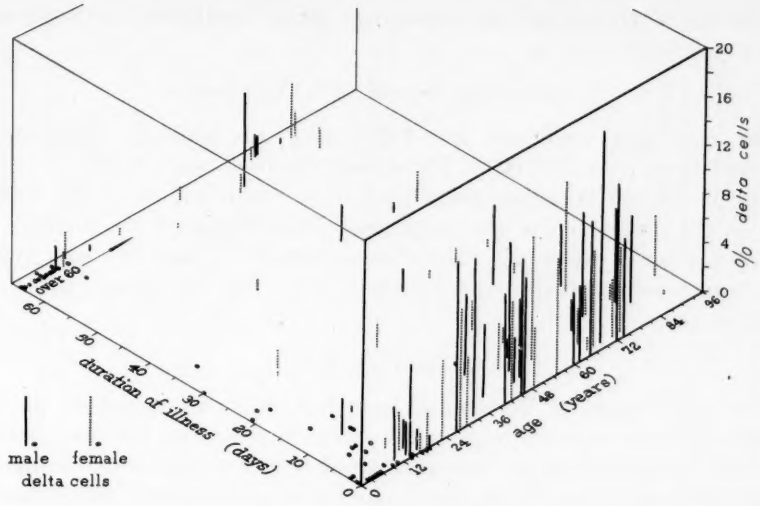

FIG. 2. Percentage of delta cells in relation to age, sex, and duration of illness.

During pregnancy, many of the alpha cells undergo a specific change. They become partially degranulated, and their outlines become indistinct. This change is most marked during the third trimester, when it becomes difficult to distinguish alpha cells from gamma cells, or even chromophobes. These partially degranulated orange-G positive cells correspond to the "pregnancy cells" first described by Erdheim and Stumme in 1909 (16). Since there are all stages of degranulation between typical alpha cells and "pregnancy cells," all cells with orange granules were included among the alpha cells. This results in the percentage of alpha cells being significantly higher in women in the third trimester of pregnancy than in nonpregnant females or in women pregnant less than six months. A normal proportion of alpha cells was found in the 2 women who died two months following delivery at term.

Beta cells (Table 4, Fig. 1)

The most significant observation regarding the beta cells is that their proportion is not influenced by age, sex or pregnancy.

Chromophobes (Table 5, Fig. 1)

The pituitaries of young children contain many chromophobes. At puberty there is a significant decrease in the proportion of chromophobes; the adult level is reached in the 16–20 age group, and there is no further change during adult life. No sex difference in the percentage of chromophobes was observed at any age. The equation for the regression of the

percentage of chromophobes versus age (sexes combined) between the limits of 0 to 20 years is:

$$\% \text{ chromophobes} = 51.52 - 1.639 \text{ (age)}.$$

The regression coefficient is -1.639, and this slope is significantly different from zero ($P = .001$). The correlation coefficient is .665.

During the last three months of pregnancy, there is a significant reduction in the proportion of chromophobes, coinciding with the increase in the proportion of alpha cells. The normal balance of these two cells types had been re-established in the 2 patients who died two months after delivery at term.

DISCUSSION

The percentages of acidophils, basophils and chromophobes in the human pituitary as reported by some earlier investigators are summarized in Table 6. The greater percentage of alpha cells and smaller percentage of chromophobes in the present study may be accounted for by the inclusion of some partially degranulated orange-G positive cells among the alpha cells. Earlier workers regarded only heavily granulated cells as chromophils, and therefore counted lightly granulated acidophils and basophils as chromophobes. In the present study, only small cells with little or no cytoplasm were considered as chromophobes.

As noted in a previous paper (18), "gamma" is a term used for a lightly-staining PAS-positive cell which probably represents the actively secreting form of both beta and delta cells. It would appear that the lightly-staining orange-G positive cells which are present in small numbers in normal glands but become very numerous in late pregnancy are related to the alpha cells in the same way as the gamma cells are related to the beta and delta cells, *i.e.*, they represent an actively-secreting form, with little storage of hormone.

The basophil counts reported by most earlier investigators (Table 6) included both beta and delta cells. Romeis (15) in 1940 was the first to describe the delta cell as a basophilic type which lacked affinity for krezofuchsin. He noted that the incidence of delta cells was extremely variable between individual glands. Herlant (8) in 1943 found cells showing metachromasia with toluidine blue. He associated their presence with the production of gonadotropin. If alcohol-fixed sections were soaked in warm water, these cells lost their affinity for toluidine blue, and the extract possessed demonstrable gonadotropic activity. Herlant believed that these cells made only follicle-stimulating hormone (FSH), whereas luteinizing hormone (LH) came from the acidophils.

Since Herlant's report it has become apparent that the luteinizing ac-

TABLE 6. PITUITARY CELL COUNTS REPORTED BY OTHER INVESTIGATORS

Investigator	Subjects	Percentages of cell types									Remarks
		Acidophils			Basophils			Chromophobes			
		In males	In females	Combined	In males	In females	Combined	In males	In females	Combined	
Rasmussen (9–12)	Children			25			17			58	
	Adults under 50 yrs.	38	45		11	6		51	48		
	Adults over 50 yrs.	34	39		11	8		55	52		
	All adults	37	43		11	7		52	50		
	Pregnant women		43			6			50*		* Pregnancy cells counted as chromophobes, which take on acidophil granulation.
Crooke & Russell (14)	Adult	29			11			60			
Halpern (17)	Children (newborn)	24	27		16	15		59	59		
	Children (older)	37	41		18	15		43	45		
Herlant (8)	Children			33			7			60	
	Adults	37	39		12	11		51	49		
	Pregnant women		23*+41**			9			27		* Normal acidophils. ** Pregnancy cells counted separately; regarded as acidophils which lose their granules and resemble chromophobes.
Floderus (13)	Adults	40			9			51			Females similar to males.

tivity which he associated with the acidophils was probably due to prolactin. As LH and FSH are glycoproteins, Pearse (19, 20) considered it likely that both hormones are produced by PAS-positive cells. Purves and Griesbach (2) and Halmi (3) demonstrated that, in the rat, thyrotropes (beta cells) and gonadotropes (delta cells) could be clearly distinguished. Later Purves and Griesbach (21, 22) reported that a further distinction could be made between FSH-producing and LH-producing gonadotropes on the basis of their spatial distribution in the gland and their reaction to extraction with trichloracetic acid.

A notable change occurs in the pituitary just before puberty; delta cells, which are absent before the age of 10, appear in small numbers. Their percentage gradually increases during adolescence, until the adult level is reached. In a review of human pituitary gonadotropins, Albert (6) concluded that little or no gonadotropin can be detected in the urine before puberty, and that the pituitaries of children contain virtually no gonadotropin. Nathanson *et al.* (23) suggested that a small amount of gonadotropin is secreted about a year before physiologic signs of puberty develop. The development of delta cells in the pituitary at about 10 years of age coincides with the appearance of appreciable quantities of gonadotropin in both the pituitary and the urine. The number of delta cells remains low during the early pubertal years. If the turnover of hormones is very rapid, many of the gonadotropin-producing cells may be partially degranulated and appear as actively-secreting gamma cells.

At the cessation of ovarian activity in women, there is a marked rise in the secretion of gonadotropins, which gradually falls off in extreme old age (6, 8). The percentage of delta cells in postmenopausal women is lower than in men of corresponding age, but the percentage of gamma cells is higher. In this stage of life, as in adolescence, the gamma cells derived from delta cells may represent actively-secreting gonadotropes.

Some earlier investigators (8, 10, 17) suggested that there are more acidophils in females than in males. In our study, the only significant sex differences in the pituitary cell counts were the greater proportion of gamma cells and the lesser proportion of delta cells in women. These differences were most marked after age 60.

The pituitary in pregnancy is hyperplastic (16). The predominant cell type in the last trimester is a lightly granulated alpha cell, which other investigators have classified as a special type of acidophil (8, 16) or chromophobe (10, 24). It seems likely that this change is related to increased production of prolactin (25, 26) and possibly growth hormone. A negative finding of equal importance is the absence of delta cells after the early weeks of pregnancy. Herlant (8) found no gonadotropin in the pituitary glands of pregnant women. Presumably gonadotropin production in these

cases is suppressed by the high estrogen levels in the circulation. In our study, the failure of the delta cells to reappear in the 2 patients who survived for two months after delivery at term may be accounted for by the long duration of the final illnesses, since it was shown previously (1) that delta cells are decreased in chronic illnesses.

Thus a detailed analysis of pituitary cell counts from birth to old age reveals the absence of delta cells from the pituitaries of children under 10 years of age and women in the last two trimesters of pregnancy. This lack of delta cells appears to be related to the virtual absence of gonadotropin in these pituitary glands, and is the basis for our contention that the delta cell in the human makes gonadotropin.

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EVALUATION OF THE PITUITARY-ADRENAL AXIS IN SIMPLE HIRSUTISM*

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ABSTRACT

Pituitary-adrenal relationships were studied in 63 women with simple hirsutism, by means of urinary 17-ketosteroid and corticoid determinations under control conditions and during maximal stimulation with ACTH and minimal suppression with prednisolone. The data were analyzed by various statistical techniques including a matrix of simple correlations. Involvement of the adrenal cortex in this clinical disorder was confirmed by several functional parameters. It appears that the adrenals of hirsute patients may be more sensitive to fluctuations in endogenous ACTH than are the adrenals of normal subjects, and that such hypersensitivity is largely confined to the ketosteroid-producing mechanism. Several features of the statistical approach to adrenal function tests, as well as the utility of the correlation matrix are discussed.

THE frequent association of increased urinary 17-ketosteroid excretion with "idiopathic hirsutism" in women has been noted for a long time. Recent studies have been directed toward identification of the source and the chemical structure of these compounds. Functional evidence of their adrenal origin was adduced from the suppressive effect of cortisone administration (1, 2) and qualitative studies (3) showed that both $C_{19}O_2$ and $C_{19}O_3$ steroids, but predominantly the former, were produced. More detailed analysis (4,5) revealed that the excretion of androsterone and etiocholanolone was often elevated as much as 300 per cent over normal, to levels characteristic of adult males. The modest increase in the excretion of 11-oxygenated 17-ketosteroids was attributed in part to the metabolism of hydrocortisone and in part to the metabolism of increased amounts of 11-hydroxy-Δ^4-androstenedione. It was concluded that the biochemical abnormality was largely confined to an overproduction of adrenal androgens and that corticosteroid synthesis was involved only to a minor degree. Increased excretion of dehydroisoandrosterone, possibly of adrenal origin, has also been observed (2, 6) but this finding has been inconstant and un-

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related to the levels of excretion of the other 17-ketosteroids (4, 7, 8, 9). Another interesting feature of the urinary steroid pattern in some hirsute women is the elevated output of certain C_{21} steroids, presumed to be pregnanediol and pregnanetriol, particularly after stimulation with adrenocorticotropin (ACTH) (10). The chemical identity of the increased pregnanetriol has been established (11).

There are some very puzzling aspects to the adrenal derangement associated with idiopathic hirsutism. It has been noted that a course of suppressive treatment with a corticosteroid may result in an apparently permanent correction of the biosynthetic abnormality (2, 12). Recently it has been suggested that stressful emotional experiences may be a precipitating factor in androgenic adrenal dysfunction associated with hirsutism (8). That other forms of trauma can also provoke hirsutism was clearly demonstrated in some of the surviving victims of the Coconut Grove fire in Boston.

In view of the new perspectives that these observations have provided, an investigation of the pituitary-adrenal mechanism was undertaken in a series of patients with hypertrichosis.

MATERIALS AND METHODS

Sixty-three women with a primary complaint of hypertrichosis were studied. The individual subject's own conviction as to the existence of hypertrichosis was accepted, and no effort was made to select cases conforming to an arbitrary, quasi-objective pattern of body hair. Except for the exclusion of patients with the adrenogenital syndrome (due either to tumor or hyperplasia) or the sclerocystic ovary syndrome, the 63 cases formed a consecutive series. Sixteen of these women, all in the reproductive age, showed gross menstrual abnormalities such as intermittent amenorrhea of several months' duration. None had persistent amenorrhea. Seven additional subjects had a history of normal, cyclic menses interspersed with occasional irregularities. In contrast to patients with the adrenogenital syndrome, only 4 showed any manifestation of virilization such as clitoral hypertrophy or masculinity of body contour; in 3 additional subjects such evidence was borderline. A positive family history of hypertrichosis was obtained in 10 cases, in 8 of which it was associated with an elevated control excretion of urinary 17-ketosteroids. This frequency of association was well within the range of random coincidence ($P = 0.95$).

17-Ketosteroid determinations were performed on samples of urine after mineral-acid hydrolysis, using color development with absolute alcohol and extraction of the Zimmermann color with the amyl acetate reagent of Masuda and Thuline (13). The normal range of excretion for adult females as measured in our laboratory is 6 to 12 mg. per twenty-four hours, and for this study 12.0 mg. per twenty-four hours was arbitrarily set as the upper limit of normal. Porter-Silber chromogens were determined on chloroform extracts of urine after glucuronidase-hydrolysis, allowing color development at room temperature for eighteen hours. The normal range of excretion as measured in our laboratory is 2 to 6 mg. per twenty-four hours and for this study 6.0 mg. per twenty-four hours was set as the upper limit of normal.

The evaluation of adrenocortical function was dependent upon the measurement of

two variables—the urinary excretion of 17-ketosteroids and of Porter-Silber chromogens—under control conditions and in states of maximal stimulation and minimal suppression. Control 24-hour urine specimens were obtained over a considerable period of time in most instances, and the values analyzed statistically. In the cases studied, it appeared that mean values were the best means of estimating the true central values, and that the value for the first urine specimen was a good approximation of the mean value.

Maximal adrenocortical stimulation was produced by a single intramuscular injection of 60 clinical units of gel-ACTH. It was not possible to use the same batch of material throughout this study, and it is recognized that this is an undesirable feature of the stimulation tests. However, the administered dose appeared sufficiently large to produce maximal stimulation even if the actual potency varied considerably from the stated value.

For suppression of pituitary ACTH production a small dose of corticosteroid was purposely chosen. It was thought that these conditions would reveal qualitative differences in the response to endogenous ACTH which might be overpowered by large doses. Prednisolone[1] was given orally in a dosage of 7.5 mg. daily in three fractional doses, for a period of ten days. The urine specimen used for determination of "suppression" values was collected during the tenth day of treatment.

From the data obtained under these conditions, comparisons were made in terms of the actual values (*i.e.*, 24-hour steroid output under control versus test conditions) as well as in terms of absolute change (milligrams of increase or decrease compared with the control amount) and percentage change. Correlations among the 14 variables so derived were studied initially by scatter diagrams and chi-square tests to indicate gross relationships. The 6 basic and 8 derived relationships were then punched on IBM cards and the matrix of 91 simple intercorrelations $\frac{n(n-1)}{2}$ calculated in an IBM 704 computer.

All 6 basic readings were available for 51 cases. Since the computer cannot handle "missing" data, a preliminary matrix was computed for these 51 cases. In the remaining 12 cases some of the stimulation or suppression test values were not available. The assumption was made that the missing test readings bore the same relation to the control readings as did the readings obtained in the 51 cases. The formula

$$\frac{\text{Control reading for case}}{\text{Mean of all control readings}} = \frac{X}{\text{Mean of parameter in question}}$$

was used to "fill in" the missing readings, and the correlations for this matrix of 63 were then re-run. The results were virtually identical with the matrix of 51. In the subsequent discussion, all values will refer to numbers obtained for the matrix of 63 (see Appendix).

RESULTS

Control values

The individual values observed in the 63 cases are shown in Table 1. The mean 24-hour 17-ketosteroid excretion for the entire group was 15.9 ± 7.5 (standard deviation) mg. and the mean 24-hour corticoid (Porter-Silber chromogens) output was 3.7 ± 1.5 mg. There were 18 pa-

[1] We are greatly indebted to Dr. Harold Upjohn of the Upjohn Company for liberal supplies of prednisolone (Delta-cortef).

TABLE 1. EFFECT OF ADRENOCORTICAL FUNCTION TESTS ON URINARY EXCRETION OF 17-KETOSTEROIDS AND CORTICOIDS (PORTER-SILBER CHROMOGENS) IN 63 HIRSUTE WOMEN

Patient No.	Control values		ACTH stimulation test		Steroid suppression test	
	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)
1	5.4	3.4	10.0	11.5	2.8	1.2
2	11.1	2.0	14.9	—	6.8	2.2
3	8.5	3.8	—	—	5.3	2.7
4	12.0	2.0	29.5	4.7	8.5	2.6
5	7.3	1.3	13.6	4.4	10.5	2.5
6	13.1	1.8	17.2	1.8	16.6	3.1
7	18.6	3.9	28.4	11.4	12.9	2.8
8	14.0	2.5	40.0	1.4	—	—
9	13.7	3.9	36.8	29.2	3.9	1.7
10	13.9	3.3	23.5	3.3	9.2	2.8
11	13.4	1.4	27.8	2.7	4.3	3.6
12	9.4	3.6	17.6	12.0	8.0	2.1
13	10.5	3.5	31.7	34.0	5.0	2.5
14	15.0	5.4	46.5	28.9	6.9	5.4
15	11.2	3.7	—	—	—	—
16	11.9	3.0	28.5	13.6	6.1	2.2
17	11.3	3.4	13.0	7.3	12.4	2.0
18	17.5	3.5	15.9	7.1	9.7	1.5
19	14.5	3.4	15.5	2.9	6.3	2.9
20	21.6	5.8	34.7	15.6	5.5	3.5
21	15.2	3.1	14.6	2.8	8.0	4.1
22	10.5	4.8	32.5	9.0	9.4	2.4
23	15.5	2.7	21.9	14.1	3.5	1.0
24	15.0	3.1	30.6	4.0	8.3	1.8
25	9.6	3.1	13.4	9.2	1.9	0.8
26	20.5	5.0	26.5	20.0	12.7	6.6
27	12.1	2.9	13.4	6.1	6.4	2.3
28	9.9	3.6	13.2	5.9	5.1	2.5
29	20.7	5.5	33.5	8.9	6.1	2.3
30	6.8	2.6	12.5	2.3	8.2	3.7
31	21.2	5.2	19.5	7.5	8.4	3.0
32	12.2	3.3	16.9	8.1	7.3	1.7
33	19.3	3.1	43.4	10.0	11.1	4.7
34	26.3	5.5	25.2	5.7	8.4	4.7
35	22.9	5.4	76.6	10.0	12.4	2.9
36	15.4	6.6	25.2	—	12.2	7.0
37	18.3	6.2	23.2	13.1	7.8	1.7
38	25.6	6.6	69.5	14.7	6.2	4.5

TABLE 1. *Continued*

Patient No.	Control values		ACTH stimulation test		Steroid suppression test	
	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)	17-KS (mg./24 hrs.)	P-S chromogens (mg./24 hrs.)
39	27.8	5.0	29.4	14.0	11.1	3.1
40	11.0	4.0	13.4	6.8	3.3	1.5
41	23.5	2.6	45.5	4.1	12.9	1.9
42	11.3	1.8	14.9	7.9	8.2	2.7
43	14.9	7.0	14.1	14.9	6.3	4.0
44	21.3	4.2	—	—	10.6	2.5
45	12.4	4.8	13.2	9.2	6.3	4.0
46	21.0	3.4	30.8	11.9	9.8	3.6
47	19.8	3.9	30.2	7.6	9.4	2.3
48	17.0	3.8	—	—	13.2	3.2
49	12.3	1.2	—	—	5.9	2.7
50	17.2	2.3	35.1	6.8	10.4	3.1
51	14.7	2.5	25.5	5.8	3.1	2.5
52	14.0	3.3	—	—	16.7	2.2
53	7.5	3.5	10.2	11.7	6.3	2.2
54	12.5	2.9	21.2	7.4	4.9	1.8
55	18.5	2.8	—	—	15.5	2.9
56	11.9	1.7	—	—	12.9	6.0
57	14.3	2.3	25.4	5.5	11.7	3.8
58	16.9	5.6	20.0	8.5	9.1	6.1
59	17.6	2.1	28.9	5.5	7.5	1.6
60	16.0	4.7	33.8	13.2	8.1	4.3
61	60.0	6.8	54.7	6.9	18.1	6.9
62	22.7	8.2	50.4	22.0	9.1	6.2
63	14.9	2.7	—	—	13.1	3.0

tients with a normal daily 17-ketosteroid excretion of 12.0 mg. or less, and in this group the mean value was 9.8 mg. In the remaining 45 patients the daily excretion was elevated to 12.1 mg. or more, with an average of 18.3 mg. In only 6 instances was the corticoid excretion elevated above the upper normal limit of 6.0 mg. per twenty-four hours, and in these cases the values were 6.2, 6.6 (twice), 6.8 and 8.2 mg. Thus, there was no instance of marked corticoid overproduction in this group of patients.

Stimulation test

The single dose of gel-ACTH produced an average increase of 10.8 ± 10.8 mg. (73.0 ± 61.5 per cent) in ketosteroid excretion and 6.0 ± 5.8 mg. (163 ± 147 per cent) in corticoid excretion in the twenty-four hours im-

mediately following injection. In 7 instances there was no rise in corticoid excretion following the injection of ACTH, but in 4 of these there was a significant increase in 17-ketosteroid excretion. In 2 cases, corticoid excretion rose but 17-ketosteroid excretion did not.

Suppression test

The mean decrease in the 24-hour excretion of 17-ketosteroids was 7.5 ± 6.7 mg. (44.0 ± 29.2 per cent) during the suppression test, and in corticoids 0.7 ± 2.5 mg. (14.0 ± 80.5 per cent). In 5 cases, neither 17-ketosteroid nor corticoid excretion fell. In 2 instances 17-ketosteroids did not fall, but corticoids did. On the other hand, in 17 cases corticoids did not fall but 17-ketosteroids did. This cannot be explained by the proportionately larger methodological error for the narrow range of normal values for corticoid excretion in milligrams as compared to the much wider range of normal 17-ketosteroid values, as discussed subsequently.

Relation between 17-ketosteroid and corticoid excretion in the control period

The matrix of correlation coefficients is shown in Table 2. It will be seen that in the control period the coefficient of correlation between 17-ketosteroid excretion (line 1) and corticoid excretion (row 8), $r_{1,8} = 0.51$, was a significant value. This relationship was studied in detail as shown in Figure 1. The regression equation (see Appendix) was $y = 2.07 + 0.105x$. Analysis revealed that the positive correlation persisted for each subgroup when the patients were divided into those showing normal 17-ketosteroid excretion and those showing elevated 17-ketosteroid excretion in the control period. Thus in this group of patients, even though the absolute level of corticoid excretion rarely exceeded the normal range, there was statistical evidence that higher rates of ketosteroid excretion were associated with relatively higher rates of corticoid excretion. This supports the concept of adrenocortical hyperactivity with respect to overproduction of C_{21} as well as C_{19} steroids in idiopathic hirsutism.

Effects of increased stimulation of the adrenal cortex with ACTH

Correlation between ketosteroid and corticoid excretion. From the correlation matrix ($r_{2,9} = 0.30$) it appears that there was a lesser relationship between the ACTH-stimulation values than there was between the control values ($r_{1,8} = 0.51$), apparently caused by the low relationship between the absolute ($r_{3,10} = 0.31$) and the relative responses ($r_{4,11} = 0.35$) of the ketosteroids and corticoids to stimulation. This is in harmony with the well-documented observation of the greater consistency of the corticoid response as compared to the ketosteroid response in normal subjects. There are several possible explanations for the low correlation. It may be that

TABLE 2. CORRELATION COEFFICIENTS

		Mean value	Std. dev.	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]
17-Ketosteroids	Control [1]	15.9	7.5	—	0.61	0.05	−0.15	0.45	−0.86	−0.29	0.51	0.06	−0.07	−0.20	0.43	0.04	0.05
	After ACTH [2]	26.5	13.2		—	0.82	0.61	0.24	−0.54	−0.31	0.40	0.30	0.23	0.07	0.32	−0.02	0.03
	Mg. incr. [3]	10.8	10.8			—	0.90	−0.04	−0.08	−0.21	0.13	0.31	0.31	0.21	0.07	−0.07	−0.02
	Per cent incr. [4]	73.0	61.5				—	−0.16	0.09	−0.16	−0.05	0.33	0.38	0.35	−0.06	−0.05	−0.02
	After steroid [5]	8.4	3.8					—	0.07	0.59	0.08	−0.11	−0.15	−0.17	0.45	0.33	0.35
	Mg. decr. [6]	7.5	6.7						—	0.64	−0.52	−0.13	−0.01	0.12	−0.22	0.14	0.14
	Per cent decr. [7]	44.0	29.2							—	−0.36	−0.20	−0.13	−0.02	0.13	0.32	0.34
P-S chromogens	Control [8]	3.7	1.5								—	0.51	0.30	−0.07	0.41	−0.22	−0.23
	After ACTH [9]	9.7	6.4									—	0.97	0.80	0.19	−0.03	−0.10
	Mg. incr. [10]	6.0	5.8										—	0.90	0.10	0.02	−0.05
	Per cent incr. [11]	163	147											—	−0.06	0.07	0.02
	After steroid [12]	3.1	1.7												—	0.45	0.45
	Mg. decr. [13]	0.7	2.5													—	0.81
	Per cent decr. [14]	14.0	80.5														—

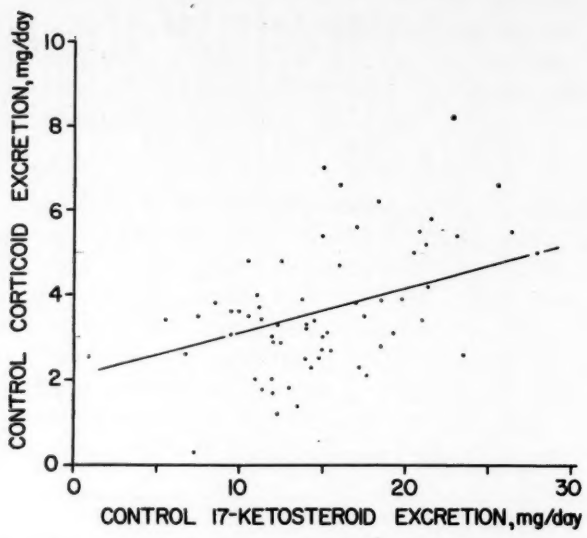

FIG. 1. Correlation between 17-ketosteroid excretion and corticoid excretion in the control period.

there is a considerable degree of autonomy in the synthesis of these two groups of steroids, either by way of separate and independent biosynthetic pathways, or by way of individual sensitivities of the enzyme mechanisms concerned with the production of one or the other type of steroid. On the other hand, the findings can also be interpreted to suggest independence of the pathways by which the adrenal steroids are metabolized to urinary 17-ketosteroids and corticoids.

Ketosteroid response. The correlation matrix reveals a significant association ($r_{1,2}=0.61$) between the control and the ACTH-stimulation excretion values for 17-ketosteroids (Fig. 2a). This is similar to the findings in a group of normal patients studied by Birke *et al.* (14) and a group of hirsute women studied by Nabarro[2] *et al.* (15). Chi-square tests and analysis of the values for the normal-control-level and elevated-control-level subgroups confirms this correlation. Thus subjects who have initially a high control excretion have a relatively high excretion during the stimulation period, and subjects who have initially a low control excretion tend to have a relatively low excretion during the stimulation period. However, the correlation matrix ($r_{1,3}=0.05$) shows that there is *no* relationship between

[2] We wish to express our appreciation to Dr. J. D. N. Nabarro for permitting us to study his original published and unpublished data.

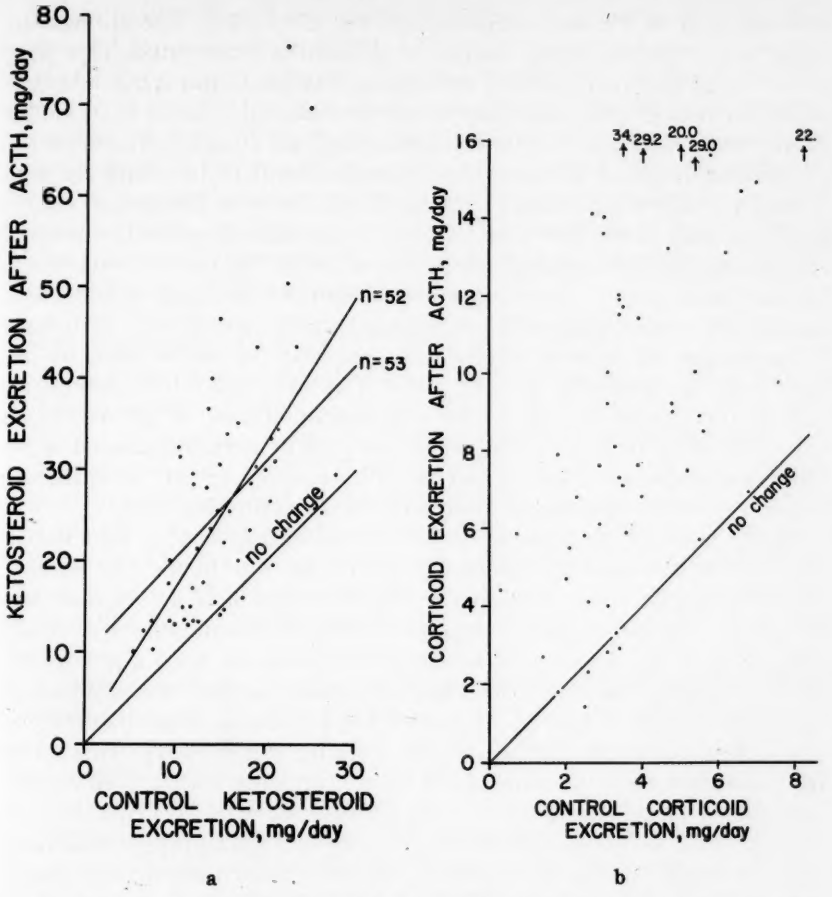

FIG. 2a. Correlation between control 17-ketosteroid excretion and 17-ketosteroid excretion during stimulation with ACTH.

FIG. 2b. Correlation between control corticoid excretion and corticoid excretion during stimulation with ACTH.

the control 17-ketosteroid output and the *absolute* increase in excretion under stimulation. Furthermore, there is no correlation ($r_{1,4} = -0.15$) between the control value and the *percentage increase* in 17-ketosteroid output under stimulation. This situation appears at first to be paradoxical, in view of the positive correlation between the control and ACTH-stimulation values. It may be resolved, however, by the following considerations. The control value C is a function of two factors, namely, the true value *c*

and the error of the determination e. Thus $C=f(c+e)$. The stimulation value S is a function of the control level plus the increment i. Thus $S=f(c+e+i)$. If there is a positive correlation between C and S but no correlation between C and i, then the correlation between C and S must be due simply to the fact that the term c appears in both C and S. Therefore the stimulation levels of 17-ketosteroid excretion tend to be relatively high in persons with a high control output simply because the control output itself was high in the first place. One may therefore question the sense of expressing (and interpreting) the results of an ACTH test in terms of the 24-hour excretion of steroid. A more meaningful approach would be to express the means and variance observed under normal and pathologic circumstances *in terms of absolute changes from the control level,* as has been done by Jenkins *et al.* (16), Jayle *et al.* (17) and others. Using this calculation combined with a two-dose administration of gel-ACTH, it was possible for Jayle *et al.* to demonstrate both hyperfunctional and hypofunctional responses with respect to 17-ketosteroid excretion—a feature which was not obtainable by other methods of evaluation (14).

In our patients the increase in ketosteroid excretion after stimulation with ACTH averaged 10.8 ± 10.8 mg. per twenty-four hours. The absence of a positive correlation between the control output of 17-ketosteroids and the absolute increase in excretion after maximal stimulation with ACTH suggests that the adrenals of hirsute patients do not have a greater *capacity* to produce ketosteroids when the control ketosteroid excretion is high than when it is normal. Moreover, the absence of a negative correlation suggests that the adrenals are not working near capacity. This leaves the possibility that the adrenals of hirsute patients with a high control excretion of 17-ketosteroids may be hyper responsive to ACTH, at least in respect to the ketosteroid-producing pathways. Such an aspect of adrenal function would have to be studied by methods other than the maximum-stimulus approach used here. The fact that only 4 subjects showed an exclusively ketosteroid response, and only 2 showed an exclusively corticoid response to ACTH indicates that such peculiarities are not appreciably more common among hirsute women than among normal subjects (19, 20).

Corticoid response. With the urinary corticoids as with the ketosteroids, there was a significant correlation ($r_{8,9}=0.51$) between the control value and the excretion after stimulation with ACTH (Fig. 2b). In patients with a high initial excretion there was a tendency toward a slightly greater absolute increase in output ($r_{8,10}=0.30$). The regression equation for the corticoid response was $y=2.1+2.04x$ with a standard error of $y=\pm 6.12$. The absolute increase in corticoid excretion averaged 6.0 ± 5.8 mg. Two patients showed a response greater than 2 standard deviations over the mean, although in both instances the *control* excretion was well within

normal limits. These cases appear to represent instances of a heightened capacity for corticoid production. The percentage increase was not correlated ($r_{8,11} = -0.07$) with the control excretion; this finding is similar to that observed for the increment in ketosteroid excretion. A chi-square analysis showed, furthermore, that patients with an elevated control excretion of 17-ketosteroids showed a more intense corticoid response to ACTH than did patients who had a normal control excretion of 17-ketosteroids (note also that $r_{3,10} = 0.31$). This emphasizes further that the elevated control excretion of 17-ketosteroids in hirsutism is associated with adrenocortical hyperfunction.

Effects of minimal suppression of endogenous ACTH

Correlation between ketosteroid and corticoid excretion. The degree of correlation between the basal excretions of 17-ketosteroids and corticoids ($r_{1,8} = 0.51$) was maintained even when the adrenals functioned under a subnormal amount of ACTH stimulation ($r_{5,12} = 0.45$). This constitutes a third line of functional evidence implicating the adrenals as the source of elevated 17-ketosteroid excretion in hirsute women. As in the case of elevated excretion during stimulation with ACTH, there was little relation between the *changes* in ketosteroid and corticoid excretion ($r_{6,12} = -0.22$) during partial suppression of endogenous ACTH with prednisolone.

Ketosteroid response. Urinary ketosteroid excretion fell during prednisolone therapy in 52 of the 59 cases in which the test was performed. The decrease averaged 7.5 ± 6.7 mg. (44.0 ± 29.2 per cent). There was a substantial correlation ($r_{1,5} = 0.45$) between the control and suppression excretions, and in this instance (as contrasted to the effect of ACTH stimulation) there was a very high correlation ($r_{1,6} = -0.86$) between the control excretion and the absolute change in milligrams. This is illustrated in Figure 3. Moreover, a strong correlation also existed ($r_{6,8} = -0.52$) between the control *corticoid* excretion and the change in 17-ketosteroids. The correlation was much weaker on a percentage basis ($r_{1,7} = -0.29$; $r_{7,8} = -0.36$). For the assessment of response to this test, therefore, regression equations and fiducial limits are necessary, in contrast to the ACTH test, in which the mean change in excretion $\pm$its confidence limits can be used. The regression equations were $y = 4.9 + 0.23x$ (S.E., $y = \pm 3.3$) for control versus suppression ketosteroid excretion, and $y = -5.0 + 0.77x$ (S.E., $y = \pm 3.4$) for control ketosteroid excretion versus absolute change during suppression. The latter relationship together with the ± 95 per cent confidence limits is shown in Figure 3.

These correlations suggest a biologically interesting hypothesis, namely, that in hirsute women with elevated ketosteroid excretion there is as high

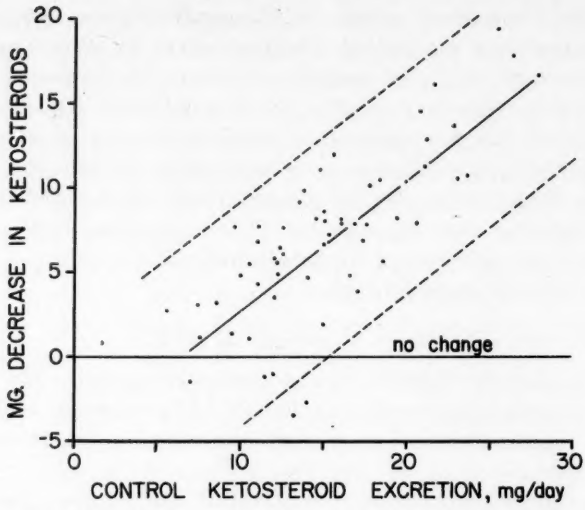

FIG. 3. Correlation between the control excretion of 17-ketosteroids and the absolute change in milligrams during suppression of endogenous ACTH with prednisolone.

or higher sensitivity to a change (in this case, a drop) in the level of ACTH stimulation as in hirsute women with normal ketosteroid excretion. This is the reverse of the relationship characteristic, for example, of Cushing's syndrome, and indicates that the functional adrenal abnormality in hirsutism may be hypersensitivity to, rather than independence

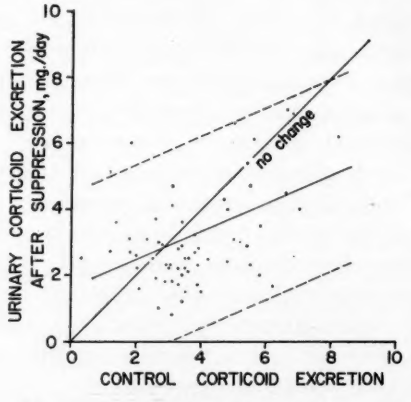

FIG. 4. Correlation between control corticoid excretion and corticoid excretion during suppression of ACTH with prednisolone.

from changes in ACTH levels. Such an hypothesis would be in harmony with suggestions that emotional stresses may trigger the development of hirsutism, and also with the curious observation of Jailer *et al.* (2) confirmed in a single case by Kappas *et al.* (12) and noted also in the present studies, that a brief course of steroid suppression may result in a permanent lowering of the overproduction of adrenal 17-ketosteroids to normal levels.

Corticoid response. There was some correlation (Fig. 4) between the control excretion of corticoids and that observed during suppression of endogenous ACTH with prednisolone ($r_{8,12}=0.41$), but very little association between the control output and the absolute or percentage change ($r_{8,13}=-0.22$; $r_{8,14}=-0.23$). This situation is evidently quite different from that observed with the ketosteroids. Since the absolute change in ketosteroid excretion was closely related to the control levels of both ketosteroid and corticoid output, whereas the change in corticoid excretion was not, the inference is that the hypothesized sensitivity of the adrenal to changes in the level of ACTH is largely confined to the 17-ketosteroid-producing mechanism.

DISCUSSION

These studies provide several lines of dynamic evidence supporting the concept of the adrenal origin of the elevated 17-ketosteroid excretion in idiopathic hirsutism. Although the predominant derangement seems to be in the androgenic component of the adrenal secretion, there is nevertheless a definite correlation between the increased productions of ketosteroid and corticoid. In this study it could be demonstrated only by statistical techniques, since the excretion of corticoids remained within the normal range in nearly all instances. In addition, it appeared that patients with elevated ketosteroid excretion showed a greater output of corticoid in response to a maximal ACTH stimulus than did patients with a normal excretion of ketosteroids. It was also observed that the correlation between ketosteroid and corticoid excretions in the control period was maintained when the adrenals functioned under subnormal levels of endogenous ACTH stimulation produced by the administration of prednisolone.

The characteristics of this functional adrenal derangement are of great interest. It appears from these as well as from many previous studies that there is considerable independence of the ketosteroid and corticoid responses to maximal ACTH stimulation. Whether this is due to effects on individual synthetic pathways or on the routes of peripheral metabolism of the adrenal steroids is as yet undecided. The changes in the urinary steroid excretion during the prednisolone suppression test show clearly that patients with relatively high control levels of ketosteroid and cor-

ticoid excretion tend to respond with a greater fall in output than do patients with normal control levels of excretion. These as well as other aspects of the study suggest the hypothesis that the adrenals in these hirsute women may be more sensitive to small fluctuations in endogenous ACTH output than are the adrenals of normal women, and that this hypersensitivity may be largely confined to the ketosteroid-producing mechanism. This phenomenon could not be demonstrated adequately with our ACTH tests because the maximal nature of the stimulus tended to obscure the quantitative details of the response. On the other hand, the maximal ACTH stimulation did serve to demonstrate that patients with elevated ketosteroid excretion do not appear to have a greater capacity to produce steroid than do patients with normal control levels of ketosteroid output.

The characteristics of the response to ACTH stimulation and prednisolone suppression show several features of statistical interest. Although a correlation between the control and the ACTH-test excretion of steroids was confirmed, it was found that this correlation did not survive when the data were examined in terms of the absolute or percentage change from normal. On this basis it would appear to be inadvisable to express the results of ACTH tests in terms of 24-hour steroid output, using regression equations for the determination of normal response. It is much simpler, and much more logical to express the results in terms of the absolute change from control values, and to calculate the means and variance for normal and abnormal populations, as Jayle *et al.* and others have done. By contrast, the results of the suppression tests show a strong correlation with the control excretions, both in terms of actual values and in terms of absolute change from control level. Under these conditions, the regression equation type of analysis is requisite. Studies based on ratios or percentage changes (20) are far less desirable, since the distributions on which fiducial limits are based may be far from Gaussian in the case of such ratios.

The statistical approach employed in these studies warrants some comment. The original analysis was carried out by means of scatter diaggrams and chi-square tests to indicate gross relationships for the data as a whole and for certain subgroups. This allowed us to specify and study these subclasses individually. Linear regressions were calculated under circumstances where a functional relationship was specified *a priori*. This type of analysis permits a description of the data by means of a straight line, and is useful for prediction of y, given x; in addition it is easy to determine if b is significantly different from zero. However, this type of presentation is very sensitive to extreme values which are concealed in the computations. Figure 2 illustrates the profound change in the regression line ($n = 53$) which results when a single extreme value (Case 61) is omitted ($n = 52$). It is a noteworthy contrast that this deletion does not

affect the standard error of y appreciably. Analysis of a matrix of simple correlations permits a much deeper exploration of the data. It is not only possible to test the strength of hypothesized associations and presumed nonassociations but also to discover associations of unexpected strength which require further inquiry and explanation. The adaptability of this approach to automated computation is a distinct advantage.

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APPENDIX

Parameters from regression analysis

Formulas used:

$$a = \frac{(\text{Sum } X^2)(\text{Sum } Y) - (\text{Sum } X)(\text{Sum } XY)}{N(\text{Sum } X^2) - (\text{Sum } X)^2}$$

$$b = \frac{N(\text{Sum } XY) - (\text{Sum } X)(\text{Sum } Y)}{N(\text{Sum } X^2) - (\text{Sum } X)^2}$$

$$\text{S.E.} = \sqrt{\frac{(\text{Estimated } Y - \text{Actual } Y)^2}{N}}$$

Title of:

Y	X	a	b	$S.E._Y$	N
Stim. 17K	Contr. 17K	10.11	1.04	11.14	53
Stim. 17K	Contr. 17K	1.46	1.63	10.8	52 (Fig. 2a)
Suppr. 17K	Contr. 17K	4.89	0.23	3.33	60
Mg. decr. 17K	Contr. 17K	−4.96	0.77	3.38	60
Stim. P-S	Contr. P-S	2.07	2.04	6.12	51
Suppr. P-S	Contr. P-S	−1.57	0.43	1.50	61
Mg. decr. P-S	Contr. P-S	−1.57	0.57	1.50	61

JCEM

THE SYNDROME OF CONGENITAL HYPOTHYROIDISM WITH DEFECTIVE DEHALOGENATION OF IODOTYROSINES. FURTHER OBSERVATIONS AND A DISCUSSION OF THE PATHOPHYSIOLOGY

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ABSTRACT

Observations are presented on 3 patients with congenital hypothyroidism and defective dehalogenation of iodotyrosines. In one, the inability of thyroid tissue to deiodinate diiodotyrosine was demonstrated *in vitro*. The same patient was found to have an extremely low thyroidal iodine content. A trend of iodine metabolism towards a more normal pattern was observed in one of the patients after a period of treatment with desiccated thyroid, but defective dehalogenation of iodotyrosines persisted. The findings in these 3 patients are contrasted with those in a fourth patient, who appeared to have congenital hypothyroidism caused by a specific defect in thyroxine synthesis. Metabolism of diiodotyrosine by thyroid tissue and after intravenous administration was found to be normal in this patient. The available evidence concerning congenital hypothyroidism with defective dehalogenation, and the significance of the principal abnormalities found in this disorder, are critically analyzed. The conclusion is reached that the defects demonstrated in this type of congenital hypothyroidism are sufficiently distinctive to permit its recognition as a separate entity.

IN 1955 circulating iodotyrosines were demonstrated in a patient with congenital goitrous hypothyroidism (1). Further features of iodine metabolism in this patient, A, were the extremely rapid uptake and release of I^{131} by the thyroid and the subnormal iodine content of the excised gland. In a cretinous brother of this patient, B, and in an unrelated patient, C, iodotyrosines and other organic iodine compounds were demonstrated in the urine. These 3 patients were unable to deiodinate intravenously administered labeled iodotyrosines (2). Thyroid tissue from Patient B had no dehalogenating activity towards diiodotyrosine *in vitro* (3).

A few apparently similar patients have been described by other investigators. Horst (4) mentions a patient with goitrous myxedema and an elevated thyroidal I^{131} uptake who excreted iodotyrosines and unidentified

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iodine compounds in the urine. McGirr *et al.* (5) in 1956 identified monoiodotyrosine (MIT) and diiodotyrosine (DIT)[1] in the urines of 2 goitrous patients and in the serum of one of them. They belonged to a large family with many cases of goitrous hypothyroidism already described in 1953 (7).[2]

Stanbury, Querido and co-workers (2, 3) developed the hypothesis that the absence of dehalogenating activity towards iodotyrosines *throughout the body* was the primary and distinctive defect in these patients. They suggested that the observed hypothyroidism might be due to the extreme want of iodine caused by this defect. Other workers have questioned the specificity of the defect of dehalogenation in the peripheral organs (8, 9) and in the thyroid (10). It was suggested that these abnormalities may be side-effects of a primary disturbance of thyroxine synthesis (8, 10), comparable to the "coupling defect" described by Stanbury *et al.* (11).

In this paper observations are reported on 3 patients with defective dehalogenation of iodotyrosines and on 1 patient who fulfilled all the criteria of the "coupling defect." In the discussion, the aforementioned hypothesis will be discussed in the light of this and other recent information.

METHODS

Radioactive iodide was administered with approximately 50 μg. of carrier iodide, either orally or intravenously. The uptake of I^{131} by the thyroid was measured with a GM tube, as described before (1). Radioactivity in urine and serum samples was measured in a well-type scintillation counter and compared with that of standards containing a suitable fraction of the dose, made up to sample volume. The serum samples were treated with trichloroacetic acid (TCA) in a final concentration of about 12.5 per cent and washed three times in 5–10 per cent TCA. In some instances, after four extractions of serum with a threefold volume of acid butanol (1), butanol-inextractable I^{131} was determined, or a complete butanol-extractable I^{131} (BEI^{131}) determination performed (12). Paperchromatograms were developed in butanol-dioxane-2*N* ammonia, 4:1:5, (BDA system) or in butanol-2*N* acetic acid, 1:1, (Bu-Ac system). Urine was chromatographed directly in amounts up to 1 milliliter. Chromatograms were cut into 1-cm. segments, and the radioactivity measured in the well-type counter.

The metabolism of labeled DIT was studied in Patients C and D. In the preparation of labeled *l*-DIT, more than 95 per cent of the labeled iodine was present as DIT. The *in vivo* metabolism of DIT in Patient D was studied by the same methods as previously used in Patient C (2). Experiments on dehalogenation by thyroid tissue *in vitro* were performed by the methods of Querido *et al.* (3), with minor changes: Tissue samples contained 0.5 Gm. of slices. Substrate DIT was added in amounts up to 100 μg. Thio-

[1] In this article the abbreviations for iodo-aminoacids and their derivatives proposed by Harington *et al.* (6) will be used.

[2] In an article which appeared after this paper was submitted, these workers, using labeled MIT, demonstrated the absence of dehalogenation by thyroid tissue in one patient, and a partial or complete peripheral defect of dehalogenation in several patients from this family (McGirr, E. M.; Hutchison, J. H., and Clement, E.: Sporadic goitrous cretinism, *Lancet* **2**: 823, 1959).

uracil (4×10^{-4} *M*) was added to all samples to prevent re-utilization of iodide. Incubation was carried out for six hours at 37°C. in closed vessels, which were aired after three hours. Control samples containing labeled DIT only, thyroid tissue only, and DIT plus heated tissue yielded only insignificant amounts of radioiodide. In the case of Patient C, a parallel experiment with beef thyroid slices was also run. Analysis was performed in two ways. The entire incubation mixture was homogenized and an aliquot (about 0.1 ml.) was chromatographed in the Bu-Ac system (Patients C and D). Samples from Patient C were also analyzed by the methods of Roche *et al.* (13).

Since it was not desirable to homogenize the entire thyroids of Patients C and D, pieces of thyroid tissue weighing approximately 1 Gm. were selected for determination of total stable iodine. For these determinations, and for assay of serum protein-bound iodine concentration and urinary iodine excretion, the methods of Barker were followed (14).

Thyroid tissue from Patient D, obtained seven days after the administration of 600 μc. of I^{131}, was analyzed for the distribution of I^{131} among the various iodine compounds. A homogenate or a saline extract thereof was incubated with pancreatin at 37°C. for twenty-four hours. Subsequent analysis was by chromatography, either of the untreated hydrolysate or of its butanol extract. Results were hardly influenced by these variations in method.

CASE REPORTS AND RESULTS

Patient C, a girl born in 1943, was a typical hypothyroid cretin. Abnormal kinetics of I^{131} metabolism and defective dehalogenation of intravenously administered labeled DIT were observed in this patient by Stanbury *et al.* in 1955. Several goitrous but euthyroid relatives of this girl were also studied (2). Thyroid substitution therapy was then started, but in full doses it resulted in restlessness and irritability. A dosage of 25 mg. daily was prescribed, but was used irregularly and finally omitted. Further growth of the goiter, which reached a size more than ten times normal, necessitated a total thyroidectomy in February 1957.

Five days prior to operation, 1 mc. of I^{131} was administered intravenously. Several samples of serum and urine collected during the first twenty-four hours after the dose (Table 1) were chromatographed both in the BDA and Bu-Ac systems. All serum samples contained large amounts of radioactive MIT, and smaller quantities of two other iodine compounds, probably identical with those found in the urine (discussed subsequently). Minor amounts of DIT and iodide were seen in the samples taken after three and six hours. Thyroxine and triiodothyronine were not found even in the 24-hour sample. By far the greatest part of the labeled iodine in the urine appeared as two compounds, x and y, which in chromatographic behavior were similar to those observed by Stanbury *et al.* (2) in their Patient B, and presumably were metabolites of MIT. Only the first urine sample (0–3 hours) contained an important amount of radioiodide. Apart from these compounds, x and y, smaller peaks of labeled iodine were visible in the Bu-Ac system, which did not correspond to any of the known iodine compounds used as markers. One of these, called z, ran between

MIT and DIT in Bu-Ac, and after elution and rechromatography in BDA, almost coincided with DIT. Identification of DIT was thus made difficult, but in all urine samples a minor peak of activity exactly corresponded to carrier-DIT in the Bu-Ac system. MIT itself could not be demonstrated in any of the samples, and therefore probably was almost quantitatively converted to one or more of the unknown substances found in the urine.

TABLE 1. PATIENT C. EXCRETION OF LABELED IODINE IN THE URINE AND TOTAL AND BUTANOL-INEXTRACTABLE RADIOACTIVITY IN THE SERUM AFTER AN INTRAVENOUS DOSE OF 1 MC. OF I^{131}

Collection period (hrs.)	I^{131} in urine (% dose)	Time after dose (hrs.)	I^{131} in serum		
			Total (% dose/L)	Butanol-inextr. (% dose/L)	Butanol-inextr. (% of total I^{131})
0– 3	8.3	3	2.69	0.33	12.2
3– 6	14.9	6	3.08	0.26	8.5
6–12	23.8	12	2.16	0.20	9.2
12–24	29.2	24	1.24	0.17	13.5
24–48	6.7				
48–72	3.2				
	86.1				

Representative examples of chromatograms of serum and urine are shown in Figure 1. The level of serum protein-bound iodine (PBI) on the first day of these studies was 0.3 μg. per 100 ml.

Two pieces of thyroid tissue weighing approximately 1 Gm. had a total iodine content between 0.25 and 0.50 μg. per Gm. The total iodine content of this thyroid, which weighed approximately 400 Gm., was therefore estimated to be between 100 and 200 μg. Slices from the thyroid of this patient did not deiodinate labeled DIT *in vitro*, even with the lowest amounts of substrate. By contrast, slices of cattle (beef) thyroid concurrently incubated showed normal dehalogenating activity (Table 2, Fig. 2). By the time of operation, radioactivity in the thyroid had decreased too much to permit analysis of its labeled compounds.

To summarize, in this patient, whose abnormal I^{131} kinetics and defective metabolism of DIT *in vivo* were already known, three further features of abnormal iodine metabolism were established: 1) a chromatographic pattern of radioactive iodine compounds in the serum and urine, com-

parable with that found in some other patients with goitrous hypothyroidism (2, 4, 5); 2) an extremely low thyroidal iodine content; and 3) inability of the thyroid tissue to deiodinate DIT *in vitro*. She therefore presented the complete picture of the syndrome originally ascribed to defective dehalogenation of iodotyrosines.

Patient D, a single woman born in 1923, had always lived in a region where iodine deficiency is rare. Because of suspected hypothyroidism, she was irregularly treated with

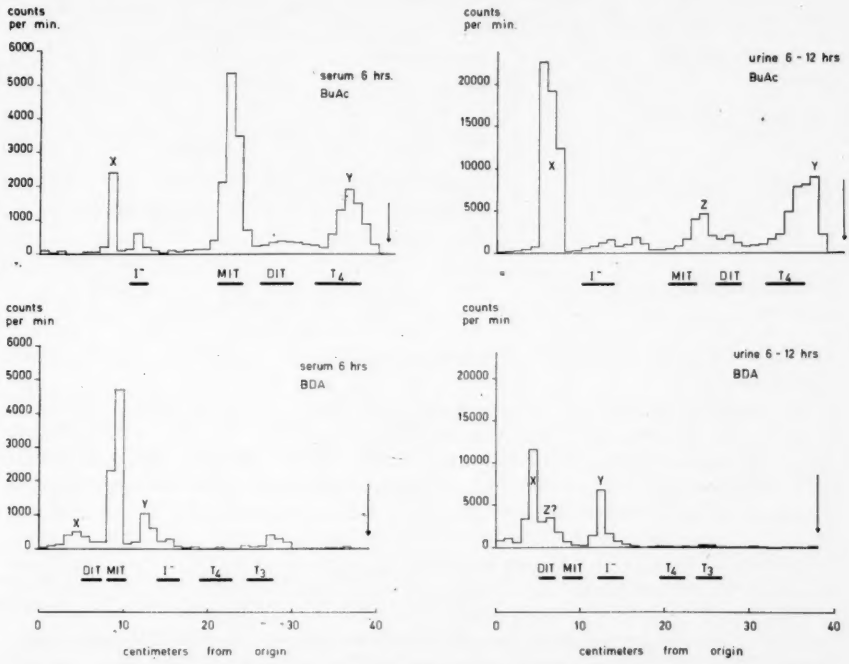


FIG. 1. *Patient C*. Chromatograms of 6-hour serum (left) and 6 to 12-hour urine (right) after an intravenous dose of 1 mc. of I^{131}. The upper chromatograms were developed in the Bu-Ac system, and the lower ones in the BDA system. The abscissa is in centimeters from the origin; the ordinate represents net counts observed per centimeter of the chromatogram. Arrows mark the solvent front. The positions of known carrier compounds are indicated below the abscissa; those of the unidentified radioactive compounds x, y and z, in the graph itself.

desiccated thyroid from the first year of life up to July 1957. A goiter had appeared at about the age of puberty (14 years). An elder brother also had a goiter. When first seen in October 1957 she seemed euthyroid. The thyroid was enlarged to four times normal size and was slightly nodular. There was slight compression of the trachea. Her height was 160 cm. Intelligence was normal. Initial findings were: BMR −6 per cent; serum

TABLE 2. PATIENT C. DEIODINATION OF I^{131}-LABELED *l*-DIT BY TISSUE SLICES

All tissue samples weighed very nearly 0.5 Gm. Varying amounts of DIT were added, but all samples contained about 0.5 μc. of I^{131} as DIT. Incubation took place for six hours at 37°C. in 3.5 ml. of Krebs-Ringer phosphate buffer containing 4×10^{-4} *M* thiouracil. A parallel experiment was run with beef thyroid slices. Recovery of labeled iodide in the analysis by the methods of Roche *et al.* (13), was 92 per cent.

Material	No. of flasks	Condition of tissue	Amount of DIT (μg.)	Recovery as I^{131}-iodide (% of I^{131} added as DIT)
Patient's thyroid	2	slices heated	2	0.9– 0.9
	2	slices	2	2.0– 2.1
	2	slices	20	1.5– 1.5
	2	slices	200	0.6– 0.9
Beef thyroid	2	slices heated	2	1.8– 1.7
	2	slices	2	62.0–58.4
	2	slices	20	56.6–36.2
Labeled DIT only	2	—	2	2.0– 2.9

PBI level, 3.0 μg. per 100 ml.; and 6-hour thyroidal uptake after 20 μc. of I^{131}, 67 per cent of the dose.

In January 1958 elaborate studies were made after intravenous administration of 60 μc. of I^{131}. Although there were still no clinical signs, the laboratory data indicated increasing hypothyroidism: the BMR ranged from −11 to −28 per cent, serum cholesterol concentration averaged 300 mg. per 100 ml., and serum PBI concentration was 1.3 μg. per 100 ml. Urinary excretion of stable iodine on two successive days was 71 and 51 μg. A therapeutic trial with triiodothyronine (25 μg. three times a day) was made for three months. Because no decrease in the size of the goiter resulted, a total thyroidectomy was performed in June 1957. Treatment was stopped for eighteen days, and a dose of 600 μc. of I^{131} was administered orally seven days before operation. The serum PBI level was 1 μg. per 100 ml. Recovery after operation was uneventful. After completion of the studies, treatment with desiccated thyroid was started. A euthyroid state and normal serum PBI concentration were maintained with a dose of 75 mg. per day.

The amounts of labeled iodine found in the thyroid, urine and serum over a period of seventeen days after the first dose of I^{131} are shown in Figure 3. A maximal thyroidal uptake of 76 per cent was found after six and twenty-four hours. Subsequent release of labeled iodine from the thyroid was slightly more rapid than normal (half-time, approximately twenty-five days). The serum PBI^{131} concentration was in the normal range, and after several days averaged 85 per cent of the total labeled iodine of the serum. Most unusual was the high, but gradually diminishing quantity of labeled iodine in the urine on the second and following days of the study.

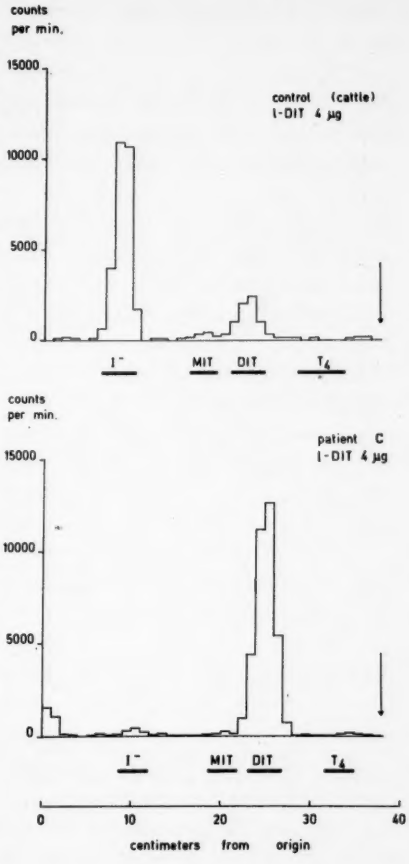


FIG. 2. *Patient C.* Dehalogenation of I^{131}-labeled *l*-DIT by thyroid slices from cattle control (above) and Patient C (below). To each sample was added 4 µg. of DIT labeled with about 1 µc. of I^{131}. The tissue samples weighed 0.5 Gm. After 6 hours at 37°C. the incubation mixtures were homogenized; 0.2-ml. amounts of the homogenates were chromatographed in the Bu-Ac system.

Values for urinary excretion and serum concentration of labeled iodine (Fig. 4) and for thyroidal uptake (not shown), which were obtained after the 600-µc. dose of I^{131}, given seven days prior to operation, almost exactly duplicated those found in the earlier study. Fractionation of serum I^{131} by several procedures demonstrated a relatively small butanol-inextractable fraction, which in all samples approximately accounted for the difference between PBI^{131} and BEI^{131} values. It made up 22 per cent of the total labeled iodine in the 24-hour sample, but only 11–14 per cent in later samples. The difference between total serum I^{131} and PBI^{131} (values obtained with Somogyi's reagent were almost the same as with TCA) amounted to 20–30 per cent of the former in most samples after twenty-four hours. It was thought to represent labeled iodide. Calculation of the renal clearance for iodide on the basis of this serum value and the observed urinary excretion yielded a value of approximately 36 ml. per minute. Chromatography, in the Bu-Ac system, of urine collected on the second and fourth day of the study showed labeled iodide only. In the BDA system the labeled iodine in serum obtained after seventy-two and one hundred and twenty hours was shown to consist mainly of thyroxine; iodide, and probably a trace of T_3, were also found. After operation, less than 0.5 per cent of the preoperative radioactivity remained in the neck. This

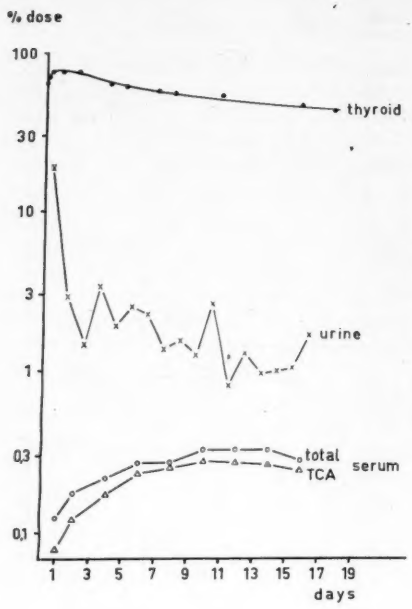


FIG. 3. *Patient D.* Measurements of radioactive iodine in the thyroid, urine and serum after an intravenous dose of 60 μc. of I^{131}. The ordinate is logarithmic and indicates the percentage of the dose of I^{131} found in the thyroid (●——●), excreted in the urine per day (×——×), or present per liter of serum (○——○). Serum PBI^{131} concentration (△——△) is also indicated. Values for excretion of I^{131} in the urine were plotted in the middle of each collection period.

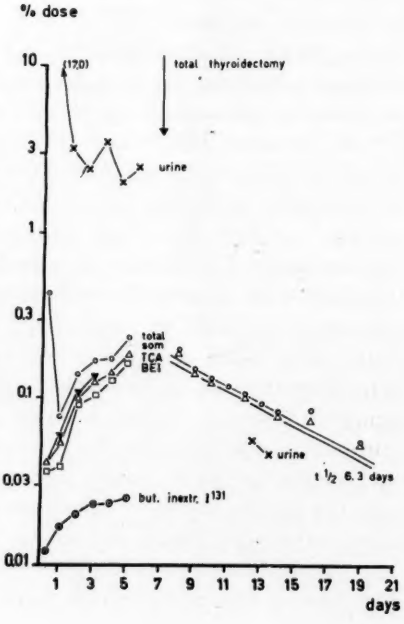


FIG. 4. *Patient D.* Radioactive iodine in the urine and serum after an oral dose of 600 μc. of I^{131}. The ordinate is logarithmic and represents the percentage of the dose of I^{131} excreted in the urine per day or present per liter of serum. Total radioactive iodine in serum (○——○) was fractionated into: a butanol-inextractable fraction (⊙——⊙), BEI^{131} (□——□) and PBI^{131} with TCA (△——△) or Somogyi's reagent (▼——▼). Urinary excretion of I^{131} (×——×) was determined daily before operation and only twice after operation. The time of total thyroidectomy is indicated by the arrow. Disappearance of I^{131} in serum after operation was approximately semilogarithmic.

permitted the disappearance rate of the remaining labeled iodine in the serum to be studied. A half-time of 6.3 days was found. I^{131} excretion in the urine fell to a small fraction of the preoperative level.

Two pieces of thyroid tissue, weighing about 1 Gm. each, had a total iodine content of 12.4 and 25.6 μg. per Gm. respectively. The iodine content of the entire thyroid, which weighed 150 Gm., was therefore estimated to be approximately 3 mg.

Several samples of thyroid tissue were incubated with pancreatin and chromatographed in both BDA and Bu-Ac systems. MIT and DIT comprised from 76 to 81 per cent of the total labeled iodine, and there was somewhat more MIT than DIT. From 8 to 10 per cent of the total I^{131} was present as labeled iodide. In no sample was more than 1 per cent of labeled thyroxine found. Triiodothyronine could not be demonstrated.

Amounts of 8 μg. and 100 μg. of I^{131}-labeled DIT were completely deiodinated when incubated for seven hours with 0.5-Gm. samples of tissue from this goiter (Fig. 5). Two weeks before operation the *in vivo* metabolism of labeled DIT had been found normal. From a dose of 100 μg. of DIT labeled with 50 μc. of I^{131}, only 2.85 per cent was excreted in the urine as DIT in four hours.

To summarize: studies of iodine metabolism in this patient with goitrous hypothyroidism revealed all the characteristics of the "coupling-defect" first described by Stanbury *et al.* (11):

1. The urinary excretion of labeled iodide was high on the second and subsequent days of the study.
2. Labeled thyroxine was found in the serum.
3. Seven days after I^{131} was given, the labeled iodine in the thyroid consisted principally of iodotyrosines, whereas hardly a trace of labeled thyroxine was found.
4. The concentration of serum PBI was low.
5. The total iodine content of the thyroid was definitely low (much lower than in Stanbury's case).

Intravenously administered I^{131}-labeled DIT was metabolized normally in this case. Thyroid tissue from the patient readily deiodinated diiodotyrosine *in vitro*.

The significance of the remarkable I^{131} kinetics needs an explanation. Taking into account that over 80 per cent of all iodide liberated was recirculated to the thyroid, the urinary excretion of, *e.g.*, 2 per cent of the dose per day as iodide, must represent the breakdown of at least 10 per cent of the dose as organic I^{131}. This amount of I^{131} cannot possibly result from the degradation of normal serum constituents at the level found (*e.g.*, 0.25 per cent of the dose per liter). It therefore must derive from iodide liberated in the thyroid by the hydrolysis and subsequent deiodina-

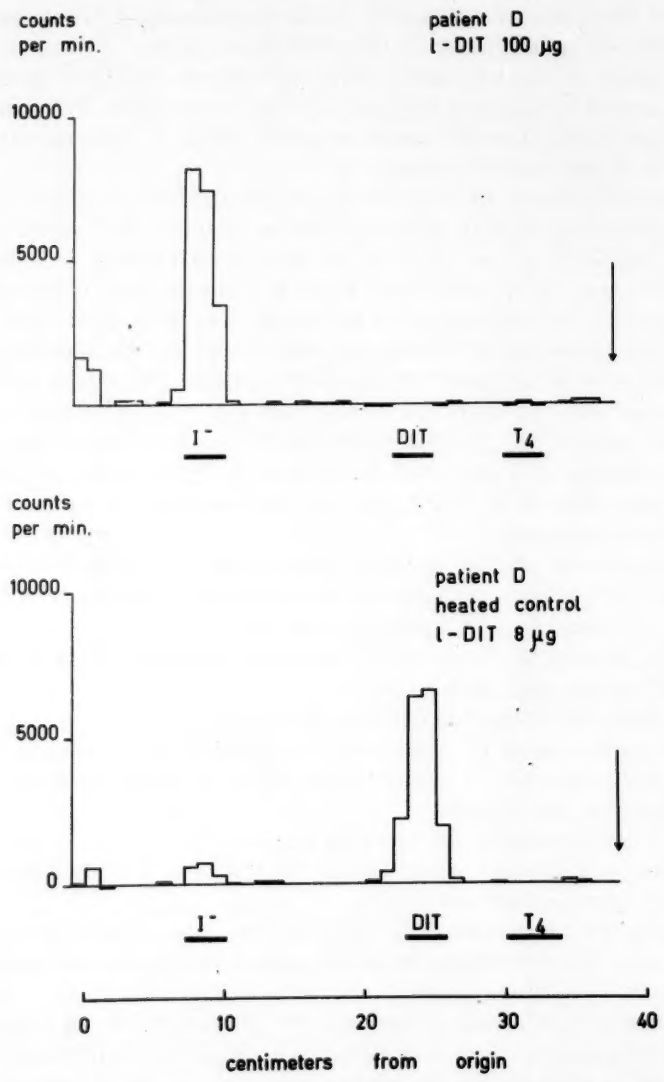


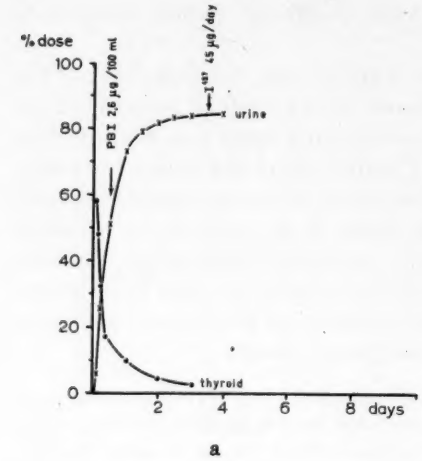
FIG. 5. *Patient D.* Dehalogenation of I^{131}-labeled *l*-DIT by thyroid slices. To the unheated sample, 100 µg. of DIT labeled with about 1 µc. of I^{131} was added as substrate. The control experiment (lower graph) was performed with heated tissue and 8 µg. of DIT.

tion of thyroglobulin, or possibly from shortlived iodine compounds secreted by the thyroid.

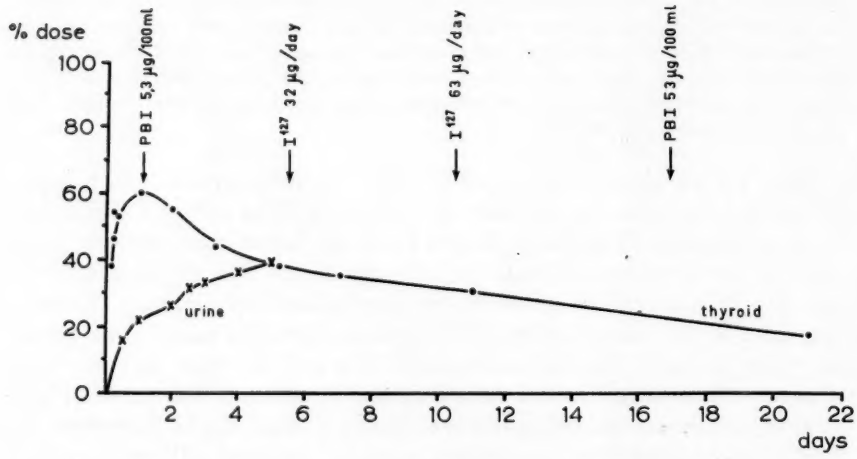
The butanol-inextractable I^{131} serum fraction (*e.g.*, 0.025 per cent of the dose per liter) could be such a compound, but to yield 10 per cent of the dose per day ought to have a half-time of about twenty-five minutes. The first possibility seems more probable. Comparison of the amount of radioiodide liberated with the concurrent radioactivity in the thyroid (*e.g.*, 65 per cent of the dose) indicates that at least 15 per cent of the thyroidal iodine stores were turned over per day, assuming that equilibration was nearly complete. Results of postoperative studies prove that these abnormal kinetics were dependent upon the presence of the thyroid and were not caused by an abnormality in the peripheral tissues.

Patient E, a male born in 1953, was the third of four children. The oldest sister, born in 1949, seemed to have normal thyroid function; but both in his elder brother (born in 1951) and in the patient, hypothyroidism was diagnosed at the age of about 1½ years. The brother was slightly mentally retarded. The younger sister, born 1956, is Patient F. The parents showed no signs of abnormal thyroid function and were not related. Treatment with desiccated thyroid was given from the age of 1½ years in dosages up to 90 mg. per day, and was stopped three weeks before the present studies were made (September 1958). By that time growth and mental development were normal. The thyroid was easily palpable.

After an oral dose of 40 μc. of I^{131}, the thyroidal uptake and urinary excretion were measured for four days (Fig. 6a). The curves were similar to those found in Patients, A, B and C (1, 2). Serum concentration after eight hours was extremely high (6.9 per cent of the dose per liter, only 21 per cent TCA-precipitable, but 95 per cent butanol-extractable). Chromatography of this serum in the BDA system showed a major peak corresponding to MIT. Smaller amounts of DIT and T_4 were probably also present. Chromatograms of urine samples collected during the first twenty-four hours showed a pattern similar to that found in Patient C. Beside two unidentified compounds probably identical with those found in Patient C, all samples, however, contained large amounts of MIT and DIT (Fig. 7). An eluate from the zone of compound x in the Bu-Ac system, when subjected to hydrolysis with 0.05 *N* HCl for one hour, was almost quantitatively converted to MIT. Only the samples of urine collected up to six hours after the dose contained a major amount of radioiodide. Identification of the small amounts of iodide in later samples was difficult because of overlap with other labeled substances. A sample of urine, collected between six and twelve hours after the dose, was analyzed by Prof. Michel, who found that compound y was mainly the acetic-acid derivative of MIT (MIT-Ac). Further results of this analysis will be reported elsewhere (15).



a



b

FIG. 6. **a.** *Patient E.* Labeled iodine in the thyroid and cumulative urinary excretion of radioactive iodine after an oral dose of 40 μc. of I^{131}. The ordinate represents the percentage of the dose of I^{131} in the thyroid (●——●) or excreted in the urine (×——×). Values for serum PBI concentration and urinary excretion of I^{127} are inscribed on the days the samples were obtained.

b. *Patient F.* Legend the same as for 6a.

The serum PBI level was 2.6 μg. per 100 ml. on the first day, and the urinary excretion of stable iodine was 45 μg. on the fourth day of these studies.

This patient therefore had the rapid thyroidal turnover of I^{131} and the chromatographic patterns of serum and urine characteristic of hypothyroidism with defective dehalogenation.

Patient F, the younger sister of Patient E, was born in 1956. In June 1957, when she was 16 months old, hypothyroidism was suspected and proved by a serum PBI determination and limited I^{131} studies. She was treated with desiccated thyroid (20 mg.

per day) for about a year. Treatment was interrupted three weeks before further I^{131} studies were made (September 1958). Growth and mental development were normal. The thyroid was slightly enlarged.

After the first dose of 10 μc. of I^{131}, administered by stomach tube, the thyroidal uptake was measured only after twenty-four hours (14 per cent of the dose). The level of total serum I^{131} after twenty-four hours was 3.5 per cent of the dose per liter. A sample of urine collected at home at nine hours contained 10 per cent of the dose. Chromatography of this urine disclosed almost no iodide, but large amounts of MIT, DIT and two unidentified compounds similar to the substances x and y found in Patient C.

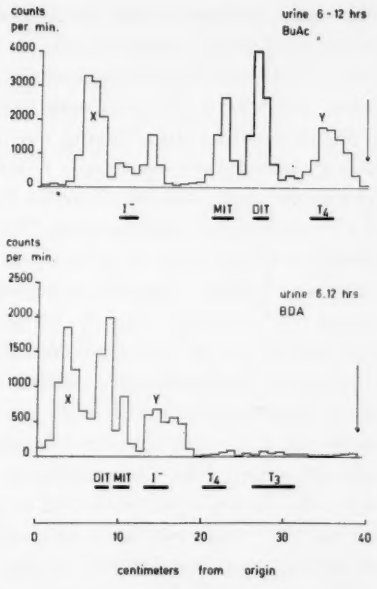


FIG. 7. *Patient E.* Chromatograms of 6–12 hour urine after oral administration of 40 μc. of I^{131}. The upper chromatogram is in the Bu-Ac system, the lower one in the BDA system.

It was therefore certain that maximal uptake in the thyroid must have occurred before twenty-four hours, and had been considerably above the 24-hour value. The serum PBI concentration was 0.5 μg. per 100 ml.

In the second study a dose of 40 μc. of I^{131} was administered orally. Subsequent measurements are shown in Figure 6b. Thyroidal I^{131} uptake was maximal after twenty-four hours (60 per cent of the dose). The labeled iodine in the gland thereafter decreased with only moderate rapidity, and was halved only after eleven days. Serum values were 0.79 per cent of the dose per liter (65 per cent TCA-precipitable) after twenty-four hours and 2.59 per cent of the dose per liter (95 per cent TCA-precipitable) after ninety-six hours. The labeled iodine of the latter sample

was over 95 per cent butanol-extractable, and by chromatography in the BDA system was found to consist mainly of T_4. Chromatography of several urine samples collected up to forty-eight hours showed a pattern similar to that found in Patient E, with the difference that iodide was the largest component up to twenty-four hours.

Values for serum PBI concentration were 5.3 μg. per 100 ml. both on the first and on the seventeenth day of these studies. Reliable 24-hour urine samples for I^{127} determination could not be collected in this infant. Stable iodine was therefore determined in two arbitrary urine samples, and the values found were multiplied by the ratio of average 24-hour creatinine excretion in this patient over the creatinine content of these samples. The approximate values for 24-hour I^{127} excretion thus calculated were 32 μg. and 63 μg. respectively.

The results clearly show that this patient had the same congenital defect as Patient E. Although the pretreatment studies were incomplete, it seems certain that during the year of treatment a marked change in iodine metabolism occurred. Comparison of the pretreatment and post-treatment values shows that in the second study the rate of turnover of thyroidal iodine was considerably lower than in the first study. Yet the defect of dehalogenation had not been cured, for iodotyrosines were excreted as before. The considerable amounts of radioiodide found in the urines up to twenty-four hours probably represented the part of the dose not taken up by the thyroid. The most probable explanation of these findings is that treatment with desiccated thyroid had permitted a store of thyroidal iodine to be built up. This may also explain why, for at least six weeks after treatment was stopped, the patient was able to maintain a normal serum PBI concentration. To judge from chromatographic findings, the serum protein-bound iodine must have consisted mainly of thyroxine. We have no data to explain the difference in I^{131} kinetics after treatment between Patient E and Patient F.

DISCUSSION

The main problem under scrutiny is whether the type of congenital hypothyroidism described in earlier publications (1–5), and also found in our Patients C, E and F, is a separate entity caused by a specific defect of the dehalogenation of iodotyrosines. We shall discuss the enzymatic mechanisms for deiodination of iodotyrosines, the defects found in this respect in the thyroid and in the peripheral tissues of these patients, and the pathogenesis of their hypothyroidism, in that order.

Enzyme studies

Roche *et al.* first demonstrated that diiodotyrosine was enzymatically deiodinated by thyroid tissue, and to a lesser degree by liver and kidney

tissue, from several animal species (13, 16). As shown by Tong *et al.*, DIT may also be deaminated, probably through transaminating enzymes, by liver and kidney preparations, but not by thyroid tissue from rats (17). This could mean that peripheral deiodination depends on previous deamination, and therefore is nonspecific, or at least different from that in the thyroid (8, 9). It seems more likely, however, that deamination is an independent and normally minor pathway of iodotyrosine degradation in some organs. In fact Stanbury has demonstrated that the enzymes involved in the deiodination of iodotyrosines are localized in the microsomes, and require TPNH as a cofactor, both in the thyroid and in the liver and kidney (18, 19). This suggests that these deiodinating mechanisms may be identical throughout the body. Although MIT is generally deiodinated more quickly than DIT, both compounds are probably degraded by the same enzyme system, since both processes require TPNH and since competitive inhibition has been found between them.

Defective dehalogenation in thyroid tissue

The absence of dehalogenating activity towards DIT was directly demonstrated in thyroid tissue from Patient B of Querido, Stanbury and coworkers (2, 3) and from our Patient C. It was found to be complete (using two analytical methods) and stood in obvious contrast to findings in toxic and nodular goiter.

This disturbance of enzymatic dehalogenation may be due to congenital absence of the enzyme system, or parts thereof, and this is the explanation we favor. Nevertheless, the possibility that a congenital abnormality would affect the conditions under which the enzyme acts has not been eliminated. Examples would be toxic inhibition of the enzyme or an abnormal protein binding its substrate. In this connection it might be tried whether serum or thyroid tissue from these patients inhibit *in vitro* dehalogenation by normal thyroid tissue. Conceivably, a primary thyroid abnormality, other than defective dehalogenation, could set unfavorable conditions for the action of the enzyme. However, it will be seen that, of the thyroid abnormalities that deserve consideration, none is likely to affect dehalogenation of iodotyrosines.

Are there reasons to suppose that in these cases the dehalogenating system fails under an excessive load of substrate? The amount of iodotyrosines liberated from thyroglobulin per unit time is not necessarily excessive in these patients, since it is probably proportional to the rate of turnover of thyroidal iodine, which is high, but also to the thyroidal iodine content, which may be extremely low. Moreover this supposition would perhaps account for the escape of some iodotyrosines from these thyroids (10), but not explain the complete absence of *in vitro* dehalogenation. It seems unlikely, however, that the capacity of the thyroidal dehalogenating

mechanisms could be exceeded to any extent, even under pathologic conditions. Since 0.5 Gm. of thyroid slices will completely deiodinate at least 100 μg. of DIT in six hours, a normal thyroid weighing 30 Gm. could deiodinate at least 24 mg. of DIT (or MIT) in twenty-four hours. This is considerably more than its total iodotyrosine content. Significantly, in a patient with the "coupling defect" (Patient D), in spite of rapid turnover of thyroidal iodine, iodotyrosines were not found in the serum or urine, and dehalogenation *in vitro* was normal.

A primary defect in thyroglobulin hydrolysis would perhaps make the thyroid secrete iodopeptides, from which the circulating iodotyrosines might derive. However, no such peptides were found. Also the complete absence of *in vitro* dehalogenation would remain unexplained, unless the unprecedented hypothesis were made, that the lack of proper substrate had caused a complete adaptive disappearance of enzymatic activity.

The supposition that defective dehalogenation might be secondary to insufficient production of thyroid hormone also meets with several objections. Patients E and F, when obviously secreting iodotyrosines, had been without substitution treatment for three weeks only, and the serum PBI values were not extremely low. Conversely, in Patient D, although the PBI concentration fell to as low a level as 1.0 μg. per 100 ml., secretion of iodotyrosines was not observed. Generally speaking, in hypothyroidism caused by a metabolic defect in the thyroid, an increase rather than a decrease of other thyroid functions is observed. This statement is exemplified by the marked degree of iodide trapping in the defect described by Stanbury and Hedge (20, 21), and by the increase in thyroidal iodine clearance and probably also in thyroglobulin hydrolysis both in the "coupling defect" and in the disorder under discussion.

Defective dehalogenation of iodotyrosines in other organs

Peripheral metabolism of labeled *dl-DIT* was first studied by Albert and Keating in treated hypothyroidism (22). Stanbury *et al.* (23) found that when labeled *dl*-DIT is given intravenously to normal subjects in doses up to 1000 μg., rapid deiodination occurs and urinary excretion of unchanged DIT is minimal. Patients A, B and C excreted the greater part of the administered *dl*-DIT (doses less than 200 μg.) in the urine as such. Results with *dl*-MIT were similar; in these patients it was not deiodinated, but excreted partly unchanged and partly as organic derivatives. In normal subjects *dl*-MIT was deiodinated, but less completely than was *l*-MIT (2). The administration of labeled MIT and DIT to these patients evidently gave rise to the same urinary iodine compounds as found after giving I^{131} as iodide.

These observations were recently criticized by Pitt-Rivers *et al.* (8), mainly on the ground that chromatography may be unreliable to demon-

strate deiodination of iodo-aminoacids. It would, however, appear that in this case the *absence* of deiodination was sufficiently demonstrated by the recovery of the administered labeled iodotyrosines and by the absence of labeled iodide from these urines. Moreover the analysis was performed by the accepted techniques in several chromatographic systems.

Fletcher *et al.* (24) showed that the capacity of peripheral deiodinating mechanisms is considerable. Deiodination of *l*-DIT was fairly complete with doses up to 10 mg., and doses of *l*-MIT up to 150 mg. were even deiodinated completely. Stanbury and Litvak (25) and Roche *et al.* (9) found virtually normal metabolism of labeled *l*-DIT in hypothyroid patients. Mosier *et al.* (26), however, observed a definite abnormality of peripheral metabolism of DIT, but not of MIT, that was seemingly corrected by treatment, in a hypothyroid cretin whose thyroid tissue deiodinated DIT normally. In a second cretin, the metabolism of both MIT and DIT was slightly abnormal, but after treatment the deiodination of DIT was normal. The significance of this observation cannot be fully estimated, since the doses employed were not mentioned.

As a whole, these data seem to eliminate two possible explanations for the defect in peripheral dehalogenation found by Stanbury *et al.*, namely, saturation of dehalogenating mechanisms (9) and hypothyroidism (8). These explanations were already improbable since, of Stanbury's original cases, Patients A and B had been thyroidectomized and Patient A had received adequate substitution treatment, before their metabolism of DIT was studied. Recent studies in our laboratory have confirmed that the peripheral abnormality persists after years of thyroid therapy, both in patients who have been thyroidectomized and those who have not.

The tendency in the literature to accept the thyroidal defect in dehalogenation as established, and to regard the corresponding peripheral defect as a secondary phenomenon, is obviously connected with the opinion that thyroidal and peripheral dehalogenation are fundamentally different processes. In that case, a combined congenital defect of both mechanisms would be most improbable. As we have seen, however, recent data suggest that these mechanisms are closely related or identical. The identification as MIT-Ac of an important urinary iodine compound found in one of our patients, and probably present in all of them, clearly suggests that in this disorder peripheral deamination of iodotyrosines proceeds normally. This implies that both in the thyroid and in the other organs there is a defect of dehalogenation only. For reasons explained, it is too early to speak of the absence of a dehalogenating enzyme.

Production of thyroxine

In all published cases of defective dehalogenation of iodotyrosines, cretinous or noncretinous hypothyroidism was found. Since in these patients

euthyroidism and a normal level of serum PBI are attained with an average dosage of desiccated thyroid, the hypothyroidism is probably caused by decreased production of iodothyronines. This in turn may be due either to an independent and specific defect (for example, the "coupling defect") or to changes in thyroidal iodine metabolism initiated by defective dehalogenation of iodotyrosines.

Stanbury, Querido and co-workers (2, 3), favoring the latter concept, suggested that secondary iodine depletion of the thyroid is the direct cause of hypothyroidism in these patients. Pitt-Rivers *et al.*, however, doubt whether any degree of iodine deficiency short of absolute could cause cretinism (8).

A low thyroidal iodine content was indeed found in Patients A (3.25 mg.) and C (0.2 mg.). It is known that in laboratory animals hypothyroidism may follow feeding on iodine-deficient diets [(27) and Kassenaar, unpublished data], but it is not certain that this may occur in man.

In simple iodine deficiency, however, an effective recirculation of iodine may be ensured by an increase in thyroidal iodine clearance. In patients with defective dehalogenation this mechanism cannot prevent the loss of the greater part of the thyroid secretion in the urine as iodotyrosines, though it may assure full utilization of dietary iodine. Even then, the production of normal amounts of thyroxine by a normally functioning synthetic mechanism would entail the loss of more iodine than an average diet containing 100–150 μg. could provide. Thus in the untreated patient the iodine content of the thyroid will probably fall to a level incompatible with effective production of thyroid hormone. The observed increased turnover of thyroidal iodine, which may be a compensatory phenomenon caused by hypothyroidism, tends to make the situation worse, by promoting loss of iodine as iodotyrosines. In this way a vicious circle may develop, in which loss of iodine causes hypothyroidism, and hypothyroidism promotes loss of iodine.

The low thyroidal iodine content found in patients with the "coupling defect" by us and by others (28) is probably explained by the fact that part of the iodide resulting from rapid intrathyroidal deiodination of iodotyrosines escapes recirculation.

But are there data to suggest that the thyroids of patients with defective dehalogenation are capable of normal thyroxine production? The circulating labeled iodothyronines found in some of them cannot be used as evidence, since this finding is thought to be compatible with a primary defect in thyroxine synthesis. The demonstration of a measurable amount of thyroxine in the thyroid of Patient A makes such a defect already improbable, but the prolonged remission with normal serum PBI values and with an almost normal turnover of thyroidal iodine in Patient F after a

period of thyroid therapy, almost proves that thyroxine synthesis itself is intact. A similar remission was seen by Zondek *et al.* (29, 30) in 2 patients with goitrous hypothyroidism after short-term treatment with massive doses of triiodothyroacetic acid. It seems probable that both forms of treatment had this prolonged effect by means of increasing the thyroidal stores of iodine. A therapeutic trial with potassium iodide might resolve this problem.

CONCLUSIONS

From the foregoing it can be concluded that there are at least two types of congenital hypothyroidism in which the defect is beyond the stage of formation of iodotyrosines. It was shown that, in the first type, specific dehalogenating mechanisms are defective in the thyroid and in the peripheral tissues. Manifestations of this defect include the appearance of iodotyrosines in blood and urine and an extremely rapid release of labeled iodine from the thyroid in the untreated patient. There are indications that thyroxine synthesis is fundamentally intact in this disorder. In the other type, thyroxine synthesis itself is presumed to be defective. The release of I^{131} from the thyroid is not extremely rapid, yet there is a remarkably high urinary excretion of radioiodide (probably caused by rapid turnover of thyroidal iodine) for many days after maximal thyroidal uptake is reached. Circulating iodotyrosines were not found in these patients and the metabolism of diiodotyrosine *in vivo* (9) and by thyroid tissue *in vitro* (26) was normal. Both defects are familial, but their occurrence together within one family has not been reported. Although there are some similarities between the two conditions, such as low thyroidal iodine content and diminished thyroxine production, the observed differences are inconsistent with a unitarian view of the nature of these defects. Accordingly they should be regarded as separate entities.

Acknowledgment

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Our thanks are also due to Prof. R. Michel, Biochimie Générale et Comparée, Collège de France, Paris, for his interest and advice in these studies.

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SOME EFFECTS OF ABNORMALITIES OF PITUITARY, ADRENAL OR THYROID FUNCTION ON EXCRETION OF ALDOSTERONE AND THE RESPONSE TO CORTICOTROPIN OR SODIUM DEPRIVATION*

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ABSTRACT

Aldosterone was measured in the urine of 16 normal subjects and 35 patients with various abnormalities of pituitary, adrenal or thyroid function. Considerable diversity was noted in the quantity of aldosterone and other adrenal steroids in the urine of individual patients with clinical signs and metabolic findings of Cushing's syndrome or mineralocorticoid excess. In infants with the salt-losing form of congenital adrenal hyperplasia, aldosterone and cortisol metabolites appeared in smaller amounts than might be anticipated in normal children under similar conditions. Although patients with hypopituitarism and hypothyroidism excreted normal amounts of aldosterone under ordinary circumstances, the administration of corticotropin failed to elicit the usual acute rise in aldosterone excretion in these cases. Certain patients with hypopituitarism failed to respond adequately to sodium deprivation. It is suggested that chronic deficiency of corticotropin or other pituitary secretion may reduce or abolish the immediate increase of aldosterone excretion usually elicited by administration of corticotropin or sodium deprivation.

ALDOSTERONE is the most active mineralocorticoid secreted by the adrenal cortex. The quantity of this hormone which appears in the urine seems, in normal subjects, to be closely related to the electrolyte intake and to the volume of body fluids (1, 2). The output of aldosterone is not consistently correlated with that of the other adrenocortical hormones. Although the other secretions of the adrenal cortex appear to be dependent primarily on the level of corticotropin (ACTH) secretion, the output of aldosterone shows considerable independence of the functional state of the anterior pituitary. The physiologic roles of corticotropin, growth hormone, and other hormones of the pituitary (and possibly of the midbrain) in the regulation of aldosterone secretion are still to be defined (3).

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Limited data exist regarding the levels of aldosterone in patients suffering from various endocrine disorders. There is considerable variation in the level of aldosterone in the urine of patients with Cushing's syndrome or with the mineralocorticoid-excess syndrome ("primary aldosteronism"), as well as in cases of congenital adrenal hyperplasia. In view of these uncertainties, additional data are presented on aldosterone excretion in patients with certain endocrine disturbances of special interest.

METHODS

Urine was collected in 24-hour specimens. The sodium and potassium content was measured by flame photometry, using a lithium internal standard. Urinary 17-hydroxycorticoids were measured after glucuronidase hydrolysis by the method of Sandberg *et al.* (4). Urinary 17-ketosteroids were measured by the method of Drekter *et al.* (5). For estimation of aldosterone output, urine was acidified to pH 1 and extracted according to method B of Axelrad *et al.* (6). This extract was washed with alkali and then fractionated by paper chromatography in toluene-propylene glycol. The region corresponding to a cortisone standard run simultaneously was cut out, eluted and concentrated. The sodium-retaining activity of this fraction was estimated by bioassay in water-loaded, adrenalectomized rats. In a few patients with increased levels of aldosterone, the quantity of aldosterone was also measured by a modification of the physicochemical method described by Neher and Wettstein (7). Estimates of urinary aldosterone obtained by the two methods agreed within the expected limits (7, 8).

RESULTS

The results of assays of aldosterone in the urine of 16 normal subjects and 35 patients with various endocrine disorders are shown in Table 1.

Healthy controls

There were 18 assays in 16 normal subjects receiving unrestricted diets. In 11 subjects, the 24-hour output of aldosterone varied between 0.8 μg. and 2.4 μg. In 2 women, aldosterone excretion was over 7.0 μg. In one of these women the sodium content of the diet was less than usually ingested; later, at a time when sodium intake was closer to the usual levels, re-assay revealed an output of 1.7 μg. per twenty-four hours. In the 3 remaining subjects no exact estimate of aldosterone excretion was possible, but the 24-hour output was at least 2.2 μg. Excluding these 3 subjects, the average output for the group was 2.3 μg. per twenty-four hours.

Pituitary disease

Three patients studied following surgical hypophysectomy excreted normal amounts of aldosterone (0.7 to 2.2 μg. per twenty-four hours). Substantially identical levels were found in 5 additional patients with anterior pituitary insufficiency, in whom the 24-hour aldosterone output was 0.7

TABLE 1. ALDOSTERONE OUTPUT IN NORMAL SUBJECTS AND IN PATIENTS WITH ENDOCRINE DISORDERS

Diagnosis	No. of cases	Aldosterone in urine (μg./24 hrs.)
Normal subjects	16	0.8 – 7.8 (avge., 2.3)
Post-hypophysectomy	3	0.7 – 2.2
Panhypopituitarism	5	0.7 – 2.4
Isolated ACTH deficiency	2	1.2 , 1.7
Acromegaly	2	2.9, 8.9
Anorexia nervosa	2	3.5, 10.9*
Myxedema (usual diet)	6	1.0 – 2.3
Myxedema (inadequate diet)	2	4.4*, 6.9*
Hyperthyroidism	1	1.6
Adrenocortical insufficiency	7	0
Cushing's syndrome	4	0 – 4.4
Adrenal virilism	1	2.4

* Sodium intake was low at the time of study.

μg. to 2.4 μg. Three assays in 2 other cases of isolated deficiency of ACTH secretion showed a 24-hour aldosterone output of 1.2 μg. to 1.7 μg.

Many patients with hypopituitarism tolerate short periods of salt-free diet without adverse effects while increased amounts of aldosterone are excreted (9). An abnormal clinical response was observed in the 2 patients listed in Table 2. Both patients had hypopituitarism of many years'

TABLE 2. OBSERVATIONS ON 2 PATIENTS WITH LONG-STANDING HYPOPITUITARISM, BEFORE AND ON THE THIRD DAY OF DIETARY SODIUM DEPRIVATION*

Patient	Diet	Urine		Serum		Aldosterone output (μg./24 hrs.)
		Sodium (mEq./24 hrs.)	Potassium (mEq./24 hrs.)	Sodium (mEq./L)	Potassium (mEq./L)	
J.S.	Normal	105	56	115	4.8	2.2
	Low-sodium	40	55	105	4.4	0.9
B.	Normal	91	50	136	4.8	0.8
	Low-sodium	66	36	129	4.5	3.3

* With a normal diet the sodium intake was approximately 110 mEq. per twenty-four hours. The "low-sodium" diet contained 35 mEq.; but only 20 to 30 mEq. of sodium was ingested on day 3.

standing. During the initial observations, there were no clinical manifestations of dehydration or sodium depletion. During a period of normal sodium intake, serum sodium concentration was very low, but sodium excretion was not excessive and aldosterone excretion was within normal limits. In response to sodium restriction, the serum sodium concentration decreased further and sodium excretion exceeded intake. The test was discontinued after three days because of the development of anorexia, weakness and hypotension. At this time, aldosterone output was even lower than during the control phase of the experiment. In the second patient, also, anorexia and weakness developed on the third day of low-sodium intake. Serum sodium concentration decreased from 136 mEq. to 129 mEq. per liter, and sodium excretion substantially exceeded intake. A small rise in aldosterone output was seen in this patient, but the level attained fell far short of the normal response (1, 2).

Two patients with acromegaly were investigated at a time when the disease appeared active. In the first case, the output of 17-hydroxycorticoids (17-OH-CS) and 17-ketosteroids (17-KS) was elevated above normal, and the output of aldosterone was also increased to 8.9 μg. per twenty-four hours. Following irradiation of the pituitary, the 24-hour excretion of aldosterone decreased to 0.9 μg., accompanied by a diminution in the output of the other adrenocortical steroids. During a subsequent pregnancy, aldosterone output rose to 32 μg. per twenty-four hours in the third trimester, a value not infrequently found in normal pregnancies of this duration (10). In the second patient, urinary 17-OH-CS excretion was slightly elevated but the outputs of 17-ketosteroids and aldosterone (2.9 μg.) were both within normal limits.

Two patients with anorexia nervosa were studied. The sodium content of the diet taken by the first patient was only modestly reduced and aldosterone output was estimated to be 3.5 μg. per twenty-four hours. In the second patient, a very low intake of food, including very little sodium or potassium, was accompanied by normal conservation of sodium and a high output of aldosterone.

Thyroid disease

Aldosterone was measured in 8 patients with thyroid deficiency whose clinical status ranged from hypothyroidism to clinically obvious myxedema. In 6, aldosterone excretion was normal (1.0 μg. to 2.3 μg. per twenty-four hours). These subjects received normal diets. In the 2 other patients, higher levels of aldosterone excretion (4.4 μg. and 6.9 μg. per twenty-four hours) were found. These 2 subjects were receiving diets of 1000 calories or less, and were excreting about 30 mEq. of sodium per twenty-four hours.

In 1 case of hyperthyroidism, excretion of aldosterone was normal.

Adrenal disease

No aldosterone was detected in the urine of 7 patients with adrenal insufficiency due to Addison's disease or following surgical adrenalectomy.

In 4 other patients who manifested the typical clinical picture of Cushing's syndrome, aldosterone excretion was measured at a time when the output of 17-hydroxycorticoids in the urine was increased. Three of these subjects had bilateral adrenal hyperplasia and excreted respectively 1.1 μg., 4.0 μg. and 4.1 μg. of aldosterone per twenty-four hours. The other patient had an adenoma of the adrenal cortex; there was no detectable aldosterone in his urine. Due to craving for salt, this patient's dietary sodium

TABLE 3. EXCRETION OF ADRENOCORTICAL HORMONES AND METABOLITES BY PATIENTS WITH A SYNDROME OF MINERALOCORTICOID EXCESS

Patient	Adrenal pathology	Urine		
		17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)	Aldosterone (μg./24 hrs.)
H.W.	Adrenocortical carcinoma	31	10–278	213
M.N.	Adenoma of right adrenal	7	17	16
H.K.	Adenoma of left adrenal	8	9	38
M.C.	Adenoma of left adrenal	11	20	55
J.T.	Adrenocortical carcinoma	29	18	0
E.P.	Adenoma of right adrenal with hyperplasia of both adrenals	67	50	0
Normal range		5–10	5–20	1–8

intake was high and sodium output was over 350 mEq. per twenty-four hours. At this level of sodium intake, little or no aldosterone may be found in the urine of normal subjects (1).

In an adult female with striking masculinization resulting from an adenoma of the adrenal cortex, the 24-hour output of 17-ketosteroids was between 800 mg. and 900 mg. A normal quantity of aldosterone was found in the urine of this patient.

The syndrome of hypertension and potassium depletion affecting renal, cardiac and muscular function has been designated "primary aldosteronism" by Conn (11). Six patients came under observation with evidence of mineralocorticoid excess due to adrenal adenoma or carcinoma (Table 3). Some of them had only a few of the symptoms originally described in this condition and some did not excrete an excessive amount of aldosterone in the urine, but all showed hypertension and potassium depletion without other evidence of hyperadrenocorticism.

Patient H.W. (reported in detail by Foye and Feichtmeir (12)) had an adrenocortical carcinoma, hypokaliemia, muscle weakness and hypertension. The output of 17-hydroxycorticoids (17-OH-CS) was elevated significantly but 17-ketosteroid (17-KS) excretion was within normal limits until terminally, when a marked rise occurred. Aldosterone output while the patient was taking a diet normal in sodium and potassium was over 200 μg. per twenty-four hours.[1]

Three of the 6 patients had an adenoma of the adrenal cortex. Patient M.N. consulted his physician because of sudden weakness of the limbs. Hypertension was noted, and further investigation disclosed hypokaliemia, alkalosis, hyposthenuria, and a consistently neutral or slightly alkaline urine. Aldosterone excretion was higher than normal during a period of unrestricted diet. Excretion of 17-OH-CS and 17-KS was within normal limits.

Patients H.K. and M.C. were young women whose only significant complaint was headache. Hypertension of moderate severity was present. Neither patient had a significant defect in the renal concentrating mechanism, and there was no history of polyuria, polydipsia or muscle weakness. The pH of the urine was between 6.5 and 7.5. The urine had a relatively high ammonium content and low titratable acidity. Serum potassium concentration was at the lower limits of normal. Potassium loading was followed by retention of considerable amounts of potassium. The urine contained abnormally large amounts of aldosterone when the patients were receiving a diet normal in sodium and potassium. Excretion of 17-OH-CS was near or above the upper limits of normal, but the excretion of 17-KS was not remarkable. Removal of the adenoma was followed in each case by a decrease in aldosterone output, reduction of the hypertension, and a rise in urinary titratable acidity to normal.

In the 2 remaining cases of the mineralocorticoid-excess syndrome, no aldosterone was found in the urine. Patient J.T.[2] had hypertension, hypokaliemia, alkalosis and muscle weakness. There was no clinical evidence of Cushing's syndrome. The urine contained increased amounts of 17-OH-CS and normal amounts of 17-KS, but no aldosterone was detected. The patient died with widespread metastases from an adrenocortical carcinoma despite prompt resection of the primary tumor. Patient E.P.[3] had severe weakness, some elevation in blood pressure, ankle edema, and a marked reduction in serum potassium concentration. The clinical picture

[1] A lower estimate of aldosterone previously reported (12) for this patient was based on inadequate data.

[2] Studied through the courtesy of the Medical Service, Veterans Administration Hospital, Oakland, California.

[3] Studied through the courtesy of the Medical Service, Mt. Zion Hospital, San Francisco, California.

did not suggest Cushing's syndrome. The blood sugar level was normal and there was no osteoporosis. There was a pronounced elevation in the output of both 17-OH-CS and 17-KS, but no aldosterone was detected on bioassay.

Urinary aldosterone was measured in 6 infants[4] with the salt-losing form of congenital adrenal hyperplasia (Table 4). All of these babies showed some degree of dehydration, and lowering of the serum sodium concentra-

TABLE 4. ALDOSTERONE EXCRETION IN 6 INFANTS WITH CONGENITAL ADRENAL HYPERPLASIA OF THE SALT-LOSING TYPE

Patient and Age	Serum		Urine	
	Sodium (mEq./L)	Potassium (mEq./L)	17-KS (mg./24 hrs.)	Aldosterone (μg./24 hrs.)
E.A., 13 wks.	124	6.4	3.1	0.6
S.W., 6 wks.	121	7.4	2.4	1.0
E.S., 2 wks.	139	8.1	2.7–5.5	0
A.D., 7 wks.	142	7.1	2.6	3.8
P.S.,* 8 wks.	128	8.4	5.4	1.1
M.B.,* 6 mos.	—	—	1.8	0
M.B., receiving ACTH	—	—	5.0	0

* These patients received either desoxycorticosterone acetate or cortisone at the time of the assay. The other subjects were not receiving corticosteroids at this time.

tion or elevation of the serum potassium concentration. In 4 cases, urine was collected for diagnostic studies prior to administration of corticosteroids. Excretion of 17-KS ranged from 2.4 to 5.5 mg. per twenty-four hours. In 1 subject, no aldosterone could be detected. Small quantities of aldosterone were found in the urine of 2 others, and 1 child excreted 3.8 μg. per twenty-four hours. Another infant, receiving suboptimal steroid therapy, excreted 1.1 μg. of aldosterone per twenty-four hours. In the urine of a 6-month-old infant adequately treated with cortisone, aldosterone was not detected either during the control observation period or after administration of ACTH.

[4] These patients were studied through the courtesy of Dr. Wilkins, Dr. Migeon and co-workers at the Harriet Lane Home, Johns Hopkins Hospital; and Dr. Carolyn Piel and Dr. Luigi Luzzati, of the Department of Pediatrics, Stanford University.

Effect of corticotropin (ACTH) on excretion of aldosterone

The dosage of corticotropin-gel was 40 units intramuscularly twice daily for two days. In each case, the urines of two control days were pooled and results were compared with those on urine collected during administration of ACTH.

Three hospital patients (Controls A., M. and D.) were studied because of unexplained weakness, but no evidence of adrenal disease was found. During administration of ACTH, the excretions of aldosterone and 17-OH-CS were increased two to three times above the control level. The excretion of 17-KS was less consistently increased (Table 5).

Two patients with anterior pituitary insufficiency and 2 with isolated deficiency of ACTH excreted very small amounts of 17-OH-CS and 17-KS, but a normal amount of aldosterone (Table 5). In 2 of these subjects there was a substantial rise in 17-OH-CS excretion following administration of ACTH for two days, but in the third patient the increase was suboptimal.

TABLE 5. EFFECT OF CORTICOTROPIN (ACTH-GEL), 40 UNITS TWICE DAILY FOR TWO DAYS, ON THE URINARY EXCRETION OF ALDOSTERONE AND OTHER HORMONE METABOLITES

Diagnosis and Patients	Control period			After ACTH		
	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)	Aldosterone (μg./24 hrs.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)	Aldosterone (μg./24 hrs.)
Controls						
A.	7.6	14.5	2.0	17.8	25.2	5.7
M.	4.6	12.3	2.5	10.1	14.2	7.7
D.	7.1	14.2	<0.8	18.1	17.7	1.9
Hypopituitarism						
P.	0.5	2.7	0.7	14.3	4.5	<0.4
S.	1.6	4.8	2.0	14.8	8.9	2.5
ACTH deficiency						
R.C.	0.9	2.0	1.0	5.9	5.3	1.0
W.	0.3	8.8	1.2	22.5*	10.1*	<0.7*
Hypothyroidism						
L.R.	4.3	8.1	1.0	11.5	11.2	1.0
G.P.	2.6	2.8	1.4	19.0	6.3	2.2
B.W.**	2.9	13.8	6.9	31.7	31.2	3.9

* After one month of ACTH injections.

** Diet low in sodium.

The output of aldosterone was not significantly affected by ACTH in any of these 3 cases. The fourth patient was studied before and after one month of ACTH administration. Although the increase in urinary 17-OH-CS and 17-KS was considerable, no significant quantity of aldosterone was detected in the urine after ACTH was given.

In 3 patients with hypothyroidism (Table 5), the excretion of 17-OH-CS was subnormal, whereas the excretion of aldosterone was normal in relation to the dietary intake of sodium. When ACTH was administered, the excretion of 17-OH-CS and 17-KS was consistently increased. Aldosterone output, on the other hand, was not significantly increased in any of the 3 cases.

DISCUSSION

The adrenal glands of normal subjects receiving normal diets secrete between 100 μg. and 400 μg. of aldosterone daily (13–15). The average 24-hour quantity of biologically active hormone recovered from the urine in this study was 2.3 μg. A similar range and mean has been obtained by other observers using bioassay procedures (16, 17), but somewhat higher values have been obtained using physico-chemical means (7, 18).

In normal subjects, sodium depletion is the most effective stimulus to increased aldosterone output. Simple dehydration and potassium loading have somewhat less striking, but definite, effects on aldosterone secretion.

Increased secretion of aldosterone into the blood of the adrenal vein is seen in rats and dogs given ACTH (19, 20). Urinary aldosterone is increased in normal men given ACTH, but after several days the output of aldosterone may fall to control levels or lower (21).

Most patients with hypopituitarism or following hypophysectomy excrete normal amounts of aldosterone (22, 23), but when ACTH is administered to patients with hypopituitarism, aldosterone output may not rise significantly. The failure of ACTH to increase the excretion of aldosterone in these patients can scarcely be related to lack of cortisol (24), since substantial amounts of cortisol metabolites appeared in the urine after stimulation. A similar lack of response was seen in a patient with "isolated" ACTH deficiency (*i.e.*, without disturbance of thyroidal or gonadal function). It seems likely, therefore, that the cause is a deficiency of corticotropin and possibly other factors acting on the adrenal cortex.

A similar defect may account for the abnormal response in hypothyroid patients. A subnormal output of 17-hydroxycorticoids and 17-ketosteroids is found in patients with hypothyroidism and myxedema. It is thought that this reflects a subnormal secretion by the adrenal cortex, even though blood cortisol levels may be maintained as a result of depression of corticosteroid metabolism (15). In these cases, administration of corticotropin is followed by a prompt increase in the excretion of 17-hydroxycorticoids,

whereas the excretion of aldosterone does not rise above the relatively normal control level. It is hard to reconcile this differential corticosteroid response in two groups of patients with the thesis that ACTH increases the supply of precursors and thereby enhances secretion of all adrenocortical hormones, unless we assume that another reaction limits the rate of aldosterone secretion under certain circumstances.

The failure of certain patients with pituitary insufficiency to withstand sodium restriction also suggests that deficiency of ACTH or other factors may impair the capacity of the adrenal cortex to increase aldosterone secretion.[5] Apparently similar patients can tolerate a low-sodium diet without adverse effects and can increase aldosterone output adequately (9). A subnormal aldosterone response and excessive sodium loss may be related to cortisol deficiency and impaired diuresis of water (24). On the other hand, little aldosterone may appear in the urine of patients with panhypopituitarism and diabetes insipidus even when the polyuria is not controlled by Pitressin (23). It seems probable that deficiencies of both cortisol and aldosterone exist in these patients, especially when increased secretion of these hormones is required.

The effect of growth hormone on aldosterone secretion is not established. A small increase in aldosterone output was seen in one of our subjects with acromegaly but not in the other, although the disease was active in both. When growth hormone from cattle was administered to an hypophysectomized patient, no substantial increase in aldosterone output occurred. Venning and co-workers (27) reported increases in aldosterone excretion following the administration of certain preparations of pituitary growth hormone.

With ordinary feedings, normal infants and young children excrete quantities of aldosterone equal to, or only slightly below the quantities found in the urine of some adults (22). Only minute amounts of 17-hydroxycorticoids and 17-ketosteroids are found in the urine of these children. When sodium intake is restricted, the rise in aldosterone output is less striking than in the adult but attains levels of 6–8 μg., or two to three times the control values (28). Of the 6 children with congenital adrenal hyperplasia (Table 4), 2 excreted no detectable quantity of aldosterone, whereas the other 4 excreted quantities of aldosterone which would be compatible with normal diet and hydration. All 6 of these infants, however, appeared dehydrated and showed evidence of sodium deficiency and hyperkaliemia. It appears, therefore, that although these patients excrete appreciable quantities of aldosterone, the amounts are distinctly subnormal in view of their clinical status. Infants with salt-losing adrenal hyper-

[5] A subnormal increase in aldosterone secretion has also been noted in hypophysectomized dogs (25, 26).

plasia fail to increase aldosterone output when given diets low in sodium (28). There can be little doubt that these children suffer from deficiencies of aldosterone and cortisol, and stimulation of the defective biosynthetic system leads to the appearance of large quantities of unusual steroids with undesired effects. The technique used in the present study did not detect the presence of the "salt-losing hormone" which has been postulated and of which certain prototypes have been isolated (29, 30). The quantities of aldosterone found would appear even less adequate if interfering substances or impaired renal function diminished the sodium-retaining or kaliuretic actions.

A considerable diversity of hormone output, clinical signs, and laboratory findings was noted in the cases of hyperadrenocorticism. There was no significant increase in aldosterone output in 4 patients with Cushing's syndrome and in 1 with adrenal virilism. However, other observers have described large amounts of aldosterone in addition to increased amounts of 17-hydroxycorticoids in the urine of certain patients with Cushing's syndrome (31, 32).

Six patients with adrenal neoplasm presented evidence of mineralocorticoid excess in the form of hypertension and various signs of potassium deficiency. In 5 of these cases, surgical removal of the abnormal adrenal tissue was followed by the return of electrolyte balance, blood pressure, kidney function and corticosteroid excretion to, or toward, the normal range. In 2 instances, the predominant hormone metabolite found in the urine gave the Porter-Silber reaction and was assumed to be derived from cortisol, although the patients showed no signs of Cushing's syndrome other than hypertension and potassium deficiency. In the other 4 cases, the urine contained increased quantities of aldosterone, together with variable amounts of other hormone metabolites. Incubation of the adrenal tissue removed at operation from 2 of these patients demonstrated that the tumor released considerably larger amounts of corticosterone and cortisol than of aldosterone (33). In these cases, the manifestations of mineralocorticoid excess probably arose, not from hyperaldosteronism alone, but from the effects of a mixture of hormones. The effects of corticosteroids are conditioned by the existing physiologic and pathologic status of the patient, and may be modified by other hormones. It seems that a syndrome of mineralocorticoid excess may develop as a result of hypersecretion of more than one hormone, and that it is not necessarily related to oversecretion of aldosterone only.

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HYPOADRENOTROPISM: THE ISOLATED DEFICIENCY OF ADRENOTROPIC HORMONE

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ABSTRACT

Two patients with an isolated deficiency of adrenotropic hormone are presented. One was a female patient, aged 58, who had a six-year history of profound weight loss, weakness and loss of axillary and pubic hair. The second was a male patient, aged 28, who suffered from weight loss, weakness and spontaneous hypoglycemic attacks. Neither patient had the hyperpigmentation characteristic of primary adrenal insufficiency and both were shown to have adrenal insufficiency that changed to a state of normal adrenal function during treatment with ACTH. Thyroidal and gonadal functions were normal. Neither patient had evidence of an intracranial tumor, and neither had received previous glucocorticoid therapy. The female patient died of adrenal insufficiency, and postmortem studies revealed small but intact adrenals, an intact pituitary and a normal hypothalamus. These patients suffered from a deficiency of circulating ACTH but the specific etiology of this deficiency is unknown.

MOST physicians consider hypopituitarism to be manifested by deficiencies of all anterior pituitary hormones (panhypopituitarism). It is not always realized that isolated deficiencies of one or more pituitary hormones may occur, even though Sheehan in his original description commented that partial deficiencies existed (1, 2), and in some clinics it is the practice to rule out pituitary deficiency if gonadotropins are demonstrable in the urine. However, hypothalamic or pituitary tumors can result in partial pituitary deficiency (3). Furthermore, Maddock and associates (4) in 1953 reported 4 patients who, without evidence of tumor, had deficiencies of gonadotropins and adrenotropins, although thyroid function was normal. Recently, it has been demonstrated (5, 6) that patients with only small remnants of anterior pituitary tissue can have normal or even hypernormal thyroid function (5, 6) despite the absence of adrenal and gonadal functions. We recently evaluated a man with a traumatic hypothalamic injury who suffered from a deficiency of adrenotropic and gonadotropic

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hormones but whose thyroid function was normal; his condition had remained undiagnosed and untreated for twenty years. In 1954, Steinberg and associates (7) reported the case of a darkly pigmented woman who had adrenal insufficiency probably resulting from an isolated deficiency of adrenotropic hormone (ACTH).

This report presents 2 patients with an isolated deficiency of adrenotropic hormone who were not hyperpigmented and who had normal thyroid and gonadal function. They seem to represent a previously undescribed pituitary deficiency syndrome.

CASE REPORTS

Case 1

D. R., a 58-year-old white female (Fig. 1) had been well during two normal pregnancies and had a normal menstrual history until the age of 38 when, following a brief period of "hot flashes," she underwent a spontaneous menopause. She was otherwise well until the age of 53 (in 1953), when she first noted mild weakness, episodes of nausea, infrequent vomiting, intermittent muscle pains in her shoulders, back, thighs and legs, and progressive weight loss totaling 32 Kg. (70 pounds) over the ensuing year. For these reasons she sought aid at the King County Hospital, where she was subsequently followed in the medical and psychiatric clinics. The presumptive diagnosis was psychoneurosis and possible anorexia nervosa.

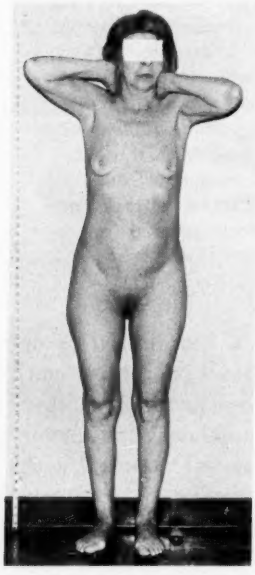

FIG. 1. Patient D. R. (Case 1) after treatment with oral hydrocortisone. She had gained 30 pounds in weight and was symptom-free. Notice lack of axillary hair and scant pubic hair, and absence of hyperpigmentation.

In December 1954, she was first admitted to the inpatient service in a state of cachexia. At the time of this admission the laboratory data shown in Table 1 were collected. Because of the normal thyroid function, the presence of gonadotropins in the urine and the decrease in the eosinophil count to zero within twenty-four hours after administration of ACTH, a diagnosis of hypopituitarism was not made, and she was discharged without specific treatment to be followed in the out-patient department.

Her symptoms continued, and in a few months she was again hospitalized with symptoms and findings similar to those described. This time hydrocortisone was prescribed in a dosage of 10 mg. twice daily, and she was discharged from the hospital. No diagnosis was made. In the next two months she gained 9.6 Kg. (21 pounds) and became free of symptoms. However, because of the earlier diagnosis of psychoneurosis and the failure to match her case with any known endocrine disease pattern, hydrocortisone was withdrawn. She immediately noted weight loss, weakness, muscle aching, and occasional nausea. After this, she failed to return for observation and took no further medication for two years.

TABLE 1. SUMMARY OF LABORATORY DATA ON PATIENT D. R.

Date	Miscellaneous laboratory data	Thyroid studies	Gonadal studies	Adrenal studies
Dec. 1954	1) Serum electrolytes: Na, 122 mEq./L; Cl, 79 mEq./L; CO_2, 22 mEq./L 2) Simultaneous urinary Cl, 60 mEq./L 3) EEG: diffuse, mild abnormality, theta type 4) Lumbar puncture: normal cells, normal protein conc. 5) Chest roentgenogram: old healed granulomatous lesion, right apex	24-hr. thyroidal I^{131} uptake, 27% (normal, 15–45%)	24-hr. urinary gonadotropin titer greater than 12 mouse units	1) Circulating eosinophils 311/cu.mm. before, and 300/cu.mm. 6 hrs. after 100 units of ACTH i.m. 2) 24 hrs. after this injection of ACTH, eosinophil count was zero 3) 24-hr. urinary 17-ketosteroids, 1.7 mg.
Feb. 1958	1) Serum electrolytes: Na, 112 mEq./L; Cl, 88 mEq./L 2) After 9α-fluorohydrocortisone, 0.2 mg. per day for 4 days, Na, 145 mEq./L	1) 24-hr. thyroidal I^{131} uptake, 29% 2) Serum PBI, 4.5 μg./100 ml. (normal, 3–8 μg.)	24-hr. urinary gonadotropin titer greater than 100 mouse units	1) Results of Robinson-Kepler-Power water excretion test (R.K.P.) (10) abnormal 2) Results of R.K.P. test normal after ACTH for 3 days 3) Eosinophil count, 300/cu. mm. before, and 240/cu. mm. after 40 units of ACTH i.v. over 8 hrs. After 2 days of ACTH i.m., eosinophil count was zero 4) Plasma 17-hydroxysteroid level, 8 μg./100 ml. before and 28 μg./100 ml. after 25 units of ACTH i.v. over 4 hrs. (probably normal)
July 1958	Hct., 37% WBC, 9,800 Urinalysis: normal urine Skull roentgenogram normal	1) 24-hr. thyroidal I^{131} uptake, 30% 2) Serum PBI, 4.5 μg./100 ml. 3) After 3 days of triiodothyronine (100 μg./day) the 24-hr. I^{131} uptake was 30%	24-hr. urinary gonadotropin titer greater than 100 mouse units	1) Results of R.K.P. water test abnormal; responded to ACTH (see Fig. 2) 2) Eosinophil count 100/cu. mm. before and 96/cu.mm. 8 hrs. after 40 units of ACTH i.v. 3) Plasma 17-hydroxysteroid level zero before, and 5.8 μg./100 ml. after 25 units of ACTH over 4 hrs. 4) 24-hr. urinary 17-hydroxysteroids, 4.8 mg. (normal 5–11 mg.) 5) Glucose-insulin tolerance test (10): blood sugar level 75 mg./100 ml. before, and 45 mg./100 ml. 30 min. after 5 units of regular insulin i.v. Patient was weak and perspiring. Blood sugar values 60, 90 and 180 mins. after admin. of glucose were 71, 76 and 73 mg./100 ml. respectively
Post mortem		Pituitary TSH content, 132 milliunits per milligram (normal)	Pituitary gonadotropin content, 0.15 unit per milligram (normal, or possibly high)	Adrenal wt. (total), 6.3 Gm. Cortices thin; no lipid deposits

She next returned in May 1957, to the psychiatric service, where a diagnosis of paranoid schizophrenia was made. Her blood pressure on admission was recorded as 90/60 mm. Hg. Without endocrine consultation she was committed to a state mental institution.

In February 1958, she was admitted to King County Hospital for the fourth time, having escaped from the state institution two to three months previously. For two weeks prior to admission she had nausea, vomiting and diarrhea. On admission, she was cachectic and weak and her blood pressure was 70/50. Laboratory data are shown in Table 1 and Figure 2. Treatment was immediately started with 9α-fluorohydrocortisone. Electrolyte levels returned to normal without other therapy. Four days after admission the serum sodium concentration was 145 mEq. per liter. Further endocrine

studies were carried out at that time. They also are listed in Table 1. Because she was recorded as an escapee from the state institution, it was necessary to return her without further evaluation. Therefore, 9α-fluorohydrocortisone (0.1 mg. per day) and hydrocortisone (10 mg. twice a day) were prescribed. The presumptive diagnosis was an isolated deficiency of ACTH.

All steroid therapy was discontinued on her return to the mental institution. After five months without medication she was readmitted to King County Hospital for further evaluation.

At this time, physical examination revealed a pale, thin woman, 147 cm. (4 feet 10 inches) in height, with no visible hyperpigmentation of the skin except over the dorsum of the feet, where the straps of sandals worn for the past month had left areas outlined

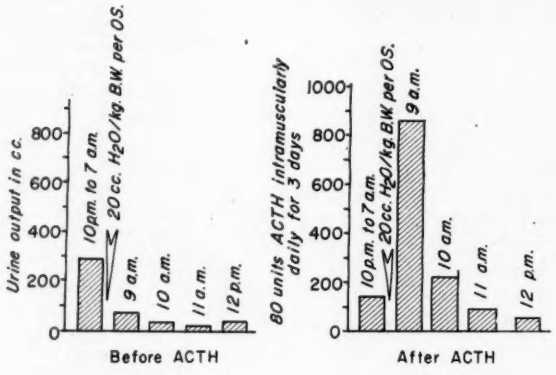

FIG. 2. Results of Robinson-Kepler-Power water excretion tests performed before and after treatment with ACTH in Patient D.R. (Case 1). This was one of several such tests which repeatedly demonstrated the response to ACTH.

with melanin. The blood pressure was 90/60, pulse 58, and respirations 16. There was no axillary hair and a very scant amount of pubic hair. The breast areolae had normal pigmentation. The heart was small. Physical findings were otherwise within normal limits.

The laboratory data are summarized in Table 1. They indicate normal thyroidal and gonadal function, with adrenal hypofunction that returned to normal after stimulation with ACTH.

The diagnosis of an isolated deficiency of ACTH appeared to be established and the patient was discharged taking hydrocortisone (10 mg. twice daily) and 9α-fluorohydrocortisone (0.1 mg. once daily). She was followed in the out-patient clinic.

During the next few months she did remarkably well except for complaints of arthralgia. She gained more than 13.5 Kg. (30 pounds), and during this period the photograph shown in Figure 1 was taken.

At this time, the test using SU-4885 [2-methyl-1, 2-bis-(3-pyridyl)-1-propanone] became available for evaluation of ACTH output (8, 9). Accordingly, hydrocortisone therapy was discontinued, and the patient was given a single intramuscular injection of desoxycorticosterone trimethylacetate for long-term maintenance of electrolyte balance. Three weeks later, when she was readmitted to the hospital, serum electrolyte levels were normal and the blood pressure was 130/80 (the level to which it had risen with hydrocortisone therapy). A control sample of urine was collected. The initial dose of 250 mg. of SU-4885 was injected intravenously and administration of 250 mg. orally every four hours was started. During this period the patient was nauseated and had two

small emeses, but her blood pressure and other vital signs remained stable until the twentieth hour of the test, when she complained of a "sore throat." Examination showed an infected pharynx and, for the first time, an elevated temperature. She arose from bed to sit in a chair, and at that instant fell to the floor. Upon examination there were no heart sounds, respirations or blood pressure, and attempts at revival were unsuccessful. Subsequently, beta-hemolytic streptococci were cultured from smears taken from the throats of patients in the same ward with pharyngeal infections. Assay of urine and blood samples taken prior to death showed no 17-hydroxycorticosteroids.

At postmortem examination an old granulomatous infection was found in the right apical lung area, and an old healed prepyloric ulcer. The heart was normal, and all vessels, including the pulmonary vessels, were patent. Examination of the endocrine glands showed a normal thyroid, and normal postmenopausal ovaries. The adrenals were small (total weight, 6.3 Gm.) and both had thin cortices. Microscopically there was diffuse hypoplasia with little evidence of lipid deposits. The pituitary weighed 646 mg. (normal) and was grossly normal. The entire pituitary gland was frozen as soon as obtained, so that hormone assays could be performed. Unfortunately, this made the gland unsuitable for accurate cell counts and differential staining techniques, so that the microscopic anatomy is not known. The pituitary contained both thyrotropic hormone (132 milliunits per milligram) and gonadotropic hormone (0.15 unit per milligram); both values were probably normal. No means were available for carrying out assay of the ACTH content. The brain, including the hypothalamus, was grossly and microscopically normal.

Summary. This pale white woman had a spontaneous menopause at age 38. She remained well until the age of 53, when she first noted weakness, nausea, muscle aches and pains, and a progressive weight loss totaling more than 32 Kg. She was admitted to the hospital on three occasions with severe hyponatremia and hypotension, which responded to adrenal steroid therapy. In contrast to the usual darkly pigmented patient with Addison's disease, she was pale. Studies of thyroidal and gonadal function repeatedly yielded normal results. Initially, adrenal function studies repeatedly yielded abnormal findings which became normal after two or more days of stimulation with ACTH. Treatment with glucocorticoids or ACTH completely relieved the symptoms. The patient died suddenly when glucocorticoid therapy was withdrawn. No 17-hydroxycorticosteroids were present in the blood or urine. The only abnormal finding at autopsy was the small size of the adrenals.

Case 2

H. B., a 28-year-old white male (Fig. 3) had been well until eight years prior to hospitalization, when he first experienced an episode of weakness, lightheadedness, profuse perspiration, nausea and vomiting. Symptoms were promptly relieved by eating. Subsequently he noted many such attacks. They were precipitated by fasting and thus were usually noted before breakfast. They increased in frequency, and occasionally he had amnesia during the attack. In 1956 he was first admitted to King County Hospital in a catatonic-like trance. At that time his blood sugar level was 30 mg. per 100 ml. He became alert and symptom-free after glucose was administered intravenously. On two

occasions at that time, ACTH failed to decrease the number of circulating eosinophils. Skull roentgenograms were normal, and an electroencephalogram showed diffuse theta activity. He was discharged to be studied further as an out-patient in the endocrine clinic. Unfortunately, he failed to return.

In January 1958, he was admitted to another hospital because he had had three days of clouded consciousness during an influenza pandemic. He spent one month in the hospital and was discharged without medication. No definite diagnosis was made. During the subsequent year he was confined to his home by the previously described attacks and a profound generalized weakness and weight loss, which by this time totaled more

FIG. 3. Patient H. B. (Case 2) after treatment with ACTH and cortisone, and when he was symptom-free. Notice normal genitalia and body hair, and absence of hyperpigmentation.

than 30 pounds. Because of this peculiar chronic illness and associated anemia, he was referred to the King County Hospital for evaluation in January 1959. Additional items of contributory history were normal erections, nocturnal emissions, and intercourse. He shaved four times weekly. There was no history suggestive of hypothyroidism.

Physical examination revealed a pale thin man, 183 cm. (6 feet) tall, who was withdrawn and hostile. His blood pressure was 100/70, pulse 80, respirations 18, and temperature 97.8°F. Although his skin was pale, the breast areolae were normally pigmented. There was no abnormal pigmentation of the buccal mucosa. Pubic and axillary hair were normal, as were the testes and prostate. The remainder of the physical findings were normal.

Laboratory studies are shown in Table 2 and Figure 4. These data indicate normal thyroidal and gonadal function, with adrenal hypofunction that returned to normal after stimulation with ACTH.

After ten days of ACTH therapy to insure that the adrenals were responsive to ACTH, an SU-4885 test (10) was performed by giving 1 gram of SU-4885 intravenously and determining the concentration of plasma 17-hydroxycorticosteroids at zero, two and six hours after the injection. The resulting values were 3, 1, and 2 μg. per 100 ml. respectively. These low levels indicated that the adrenal was not being stimulated by endogenous pituitary ACTH although the earlier studies demonstrated that the adrenals

TABLE 2. SUMMARY OF LABORATORY DATA ON PATIENT H. B.

Date	Miscellaneous laboratory data	Thyroid studies	Gonadal studies	Adrenal studies
Jan. 1959	Hct., 34% WBC, 5,000 Differential count normal except for 7% eosinophils Urinalysis: normal urine Serum electrolytes normal Hct., 42% after ACTH	1) Serum PBI, 4.8 μg./100 ml. (normal 3.5–8.0 μg.) 2) 24-hr. thyroidal I^{131} uptake: 13% before, and 28% after cortisone therapy (normal 15–45%)	1) History of normal libido & erections 2) Physical exam.: normal testes & phallus 3) 12-hr. urinary gonadotropin titer normal by mean ovarian wt. method; 96.2 mg. by Albert's method (11) (normal 17–100 mg.)	1) Results of Soffer-Gabrilove water test (12) abnormal repeatedly 2) Plasma 17-hydroxysteroid level 0.5 μg./100 ml. before, and 3 μg./100 ml. after 25 units of ACTH i.v. over 4 hrs. (Jailer test) 3) Eosinophil count 117/cu.mm. before, and 172/cu.mm. after 40 units of ACTH i.v. over 10 hrs. 4) Insulin-glucose tolerance test (Engel-Scott) (10): control blood sugar, 60 mg./100 ml; 30 mins. after 0.1 unit of insulin per kg. i.v., blood sugar, 27 mg./100 ml. Patient weak, diaphoretic; 30 mins. and 90 mins. after 100 Gm. of glucose orally, blood sugar levels were 50 and 79 mg./100 ml. respectively. 5) A 36-hr. fast resulted in hypoglycemia, with a blood sugar level of 26 mg./100 ml. 6) After 3 days of ACTH, the plasma 17-hydroxysteroid level was 2 μg./100 ml. before, and 18 μg./100 ml. after 4 hrs. of ACTH. 7) Eosinophil count dropped from 272 to 44 per cu.mm. after 8 hrs. of ACTH on the third day of ACTH therapy. 8) After ACTH therapy, the results of the Soffer-Gabrilove test were normal. 9) No hypoglycemia during fasting, when patient receiving ACTH therapy 10) After 7 days of a 250-mg. sodium diet, the concentration of urinary Na was 1 mEq./liter

would respond to exogenous ACTH. A diagnosis of an isolated deficiency of ACTH was therefore established. Glucocorticoid therapy was started and the patient quickly became free of fasting hypoglycemia. After the initial water diuresis, he began to gain weight and became active. There was also a pronounced improvement in his personality. When he was discharged he was taking 20 mg. of hydrocortisone twice daily.

At the time of follow-up examination four months later (when the photograph in Figure 3 was taken), he had no complaints and had gained 18 Kg. (40 pounds) in weight. His personality had attained normality and no further hypoglycemic episodes had occurred. He was able to undertake full-time work for the first time in several years.

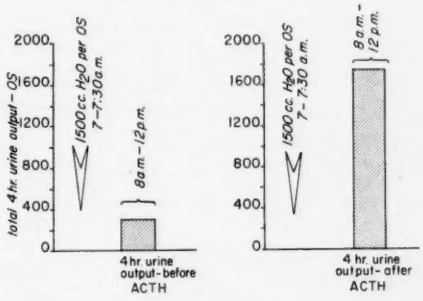

FIG. 4. Results of Soffer-Gabrilove water excretion tests performed before and after treatment with ACTH in Patient H.B. (Case 2). This demonstrates the abnormal findings before ACTH, and the change to normal after treatment with ACTH.

Summary. This 28-year-old pale, white man had an eight-year history of episodic weakness, lightheadedness, profuse perspiration, nausea, and vomiting associated with proved hypoglycemia. Thyroidal and gonadal functions were normal. Results of adrenal function studies were abnormal initially but became normal during three or more days of stimulation with ACTH. Treatment with glucocorticoids completely relieved all symptoms and resulted in an increase in appetite and a gain in weight, along with markedly favorable personality changes.

DISCUSSION

These 2 patients demonstrated normal thyroidal and gonadal function in the presence of adrenal insufficiency. The hypoadrenalism changed to a state of normal adrenal function after three days or less of stimulation with ACTH (Table 3). The patients were not hyperpigmented, in contrast to the usual patient with primary adrenal insufficiency. We con-

TABLE 3. SUMMARY OF SIGNIFICANT DATA ON 2 PATIENTS WITH AN ISOLATED DEFICIENCY OF ADRENOTROPIC HORMONE

Sex	Female	Male
Age (years)	58	28
Skin	Pale (not hyperpigmented)	Pale (not hyperpigmented)
Body hair	Deficient	Normal
Thyroid function: Serum PBI level Thyroidal 24-hr. I^{131} uptake	 4.5 μg./100 ml. 30%	 4.8 μg./100 ml. 13 to 28%
Sexual function	Gonadotropin in 24-hr. urine specimen (postmenopausal) =100 mouse units	a) History of normal libido and erections b) Genitalia normal on physical examination c) Urinary gonadotropin excretion normal
Adrenal function (initial)	a) Abnormal results with Robinson-Kepler-Power test b) Circulating eosinophil count, 100/cu. mm. before, and 96/cu.mm. after 8 hrs. of ACTH c) 24-hr. urinary 17-ketosteroids, 1.7 mg. d) Plasma 17-hydroxysteroids undetectable before, and 5 μg./100 ml. after 4 hrs. of ACTH e) Abnormal insulin tolerance curve.	a) Abnormal results with Soffer-Gabrilove test b) Circulating eosinophil count, 117/cu. mm. before, and 172/cu.mm. after 8 hrs. of ACTH c) Plasma 17-hydroxysteroid level, 0.5 μg/100 ml. before, and 3 μg./100 ml. after 4 hrs. of ACTH d) Fasting blood sugar level, 26 mg./100 ml. e) Abnormal insulin tolerance curve
Adrenal function after admin. of ACTH for 3 or more days	a) Water excretion normal b) Circulating eosinophil count, 96/cu. mm. before, and zero after 8 hrs. of ACTH	a) Water excretion normal b) Circulating eosinophil count 272/cu. mm. before, and 44/cu.mm. after 8 hrs. of ACTH c) Fasting hypoglycemia no longer present
Results of treatment	Treatment with small doses of hydrocortisone abolished all symptoms.	

cluded that they suffered from a deficiency of pituitary adrenotropic hormone. The one patient who was examined at autopsy had small, but otherwise intact adrenals. To our knowledge this syndrome of pituitary insufficiency has not been previously described. Steinberg *et al.* (7) previously reported a single patient with an isolated deficiency of ACTH, but this patient was darkly pigmented.

Thyroid function was tested by means of serum protein-bound iodine and thyroidal radioiodine uptake determinations. It is interesting that initially the male patient had a radioiodine uptake of 13 per cent, but within a week after the start of steroid treatment it had risen to 28 per cent. The reasons for this are unknown. In addition, the female patient failed to show suppression of the thyroidal radioiodine uptake during treatment with 100 μg. of triiodothyronine daily (Table 1)—an unusual phenomenon in patients with normal thyroid function. Nevertheless, the normal serum protein-bound iodine level, the normal thyroidal radioiodine uptake, the normal thyroid gland at necropsy, and the normal results of pituitary thyrotropic hormone assay, all indicated that no thyroid deficiency was present.

Gonadotropic function was shown to be normal in the female patient by repeated demonstration of high titers of urinary gonadotropins, and the demonstration of a normal pituitary content of gonadotropin post mortem. In the male patient, the history of normal sexual function, the demonstration of a normal phallus and testes on physical examination, and the normal excretion of urinary gonadotropin, all indicated normal gonadal function.

Adrenal function was shown to be deficient by the use of several parameters currently employed to test adrenal function. These included nonspecific findings such as inability to excrete water, failure to show a reduction in circulating eosinophils in response to ACTH, and a characteristic insulin-glucose tolerance curve, in addition to the specific findings of urinary and plasma steroid assays and the recently introduced SU-4885 test. In each case these results were compatible with adrenal insufficiency. Interestingly, the initial plasma 17-hydroxycorticosteroid response to ACTH (8–28 μg. per 100 ml.) in the female patient was probably normal. However, this test in our experience—*unless the results are grossly abnormal* (as in the male patient and subsequently in the female patient)—is not useful in eliminating the diagnosis of adrenal failure secondary to hypopituitarism. We have observed several patients with severe panhypopituitarism who had a low excretion of corticosteroids, but who responded fairly well to exogenous ACTH.

Our male patient had a normal distribution of body hair, and it might be concluded that this growth was supported by testicular androgens. The

female had loss of body hair, and this is probably explained by loss of her only source of androgen with failure of adrenal function. In addition, the female patient had great difficulty with electrolyte conservation and on several occasions was admitted with severe hyponatremia. This defect was readily corrected by treatment with 9α-hydrocortisone. The male patient had normal ability to conserve sodium, as demonstrated when sodium deprivation caused a drop in urinary sodium concentration to 1 mEq. per liter. Thus, one patient secreted sufficient salt-retaining steroids and the other did not. It is now known that aldosterone is secreted in surgically hypophysectomized patients, independent of ACTH stimulation (13, 14). However, certain patients with long-standing hypopituitarism are unable to secrete aldosterone in response to these normal stimuli until cortisone or its equivalent is supplied (15). We have cared for a 56-year-old woman who had panhypopituitarism for more than eight years and who had severe loss of sodium when hydrocortisone was not replaced. On two occasions when this woman with panhypopituitarism was hyponatremic and hypotensive her 24-hour urine specimens contained less than 2 μg. of aldosterone. Although the possibility needs further investigation, it seems likely that aldosterone secretion may be abnormal in long-standing deficiencies of glucocorticoids but normal in partial or short-term deficiencies.

The absence of hyperpigmentation indicates that melanocyte-stimulating hormone (MSH), a hormone identical in part to ACTH (16), was not present in excessive amounts. This suggests that ACTH was not being formed in excessive amounts. When ACTH is secreted in large amounts, as it is in primary adrenal insufficiency, melanocyte-stimulating hormone is usually secreted concomitantly in large amounts (17). However, that these patients did secrete melanocyte hormone is manifested by the pigmented breast areolae in both, and by the melanin pigmentation outlining the area of the sandal straps on the feet of the female patient. Apparently the darkly pigmented patient described by Steinberg *et al.* (7) secreted MSH in large amounts, as do patients with primary adrenal insufficiency. Certainly the absence of hyperpigmentation and the response to exogenous ACTH, associated with the finding of intact adrenals at postmortem examination, demonstrate important differences from the usual patient with Addison's disease.

The exact nature of the defect in these patients is still in question. Recent work has demonstrated in rats and possibly monkeys that certain hypothalamic lesions located anterior to the mammillary bodies and posterior to the optic chiasm are associated with abnormalities in the release of ACTH (18–21). On the basis of evidence now available, hypothalamic lesions would best explain the defect observed in these patients.

Addendum

Since this paper was written, we have been informed that Dr. Frank Tyler of the University of Utah is studying a similar patient.

Acknowledgment

We should like to thank Dr. John Bakke, Seattle Veterans Administration Hospital, for performing the plasma 17-hydroxycorticosteroid and pituitary thyrotropic hormone assays; and Dr. C. Alvin Paulsen, Pacific Northwest Research Foundation, for performing the pituitary gonadotropic hormone assays on Patient #1 and the urinary gonadotropic hormone assays on Patient #2.

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HYPERTHYROIDISM AFTER SECTION OF THE PITUITARY STALK FOR MALIGNANT EXOPHTHALMOS: REPORT OF A CASE

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ABSTRACT

In the patient discussed in this report, hyperthyroidism developed after section of the pituitary stalk and after anterior pituitary deficiency was evident from clinical examination and laboratory tests. Assays for thyrotropin in the blood at a time when signs of pituitary deficiency existed, showed the presence of a thyroid-activating substance similar to that found in other patients with hyperthyroidism.

INTRODUCTION

THYROID function has been reported to persist after total hypophysectomy (1), and the completeness of hypophysectomy is often uncertain. In a few reported cases, active hyperthyroidism occurred in patients with anterior pituitary failure (2–5). Becker (6) described 2 patients with Graves' disease who underwent hypophysectomy and whose thyroidal radioiodine uptake and serum protein-bound iodine (PBI) concentration did not fall to hypothyroid levels. Werner and associates (7) found a hyperthyroid pattern for the distribution of the iodinated compounds in serum after hypophysectomy in cases of Graves' disease. In contrast, radioactivity in the serum (after a tracer dose of I^{131}) was present almost entirely in the iodide fraction following hypophysectomy in 6 euthyroid persons. Buxton and associates (8) obtained evidence of reduced adrenal and thyroid function in 12 patients after section of the pituitary stalk for mammary carcinoma. Thyroid function improved within three months in 5 of the 6 patients examined at that time, but adrenal and gonadal function remained low for as long as eight months.

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We wish to present a case in which hyperthyroidism developed after section of the pituitary stalk for malignant exophthalmos, and persisted despite anterior pituitary failure.

CASE REPORT

The patient was a 45-year-old woman. In 1934 she had had episodes of nervousness, crying, intolerance to heat, and palpitation. The diagnosis of hyperthyroidism was made, and a subtotal thyroidectomy was performed elsewhere.

From 1934 to 1954 exophthalmos increased, and in 1954 symptoms of hyperthyroidism reappeared. From 1954 to 1957 she was treated with propylthiouracil in doses of 50 to 200 mg. daily; symptoms were not controlled by 50 mg. daily.

She was first examined at the Cleveland Clinic on September 30, 1957. The striking feature at this time was severe bilateral exophthalmos (33 mm. O.U. as measured by the Hertel exophthalmometer). There was prominent bulging of the eyelids with mild chemosis. Retraction of the lids was marked. There was slight limitation of the upward and lateral gaze. The thyroid gland was enlarged to approximately 40 Gm. The basal metabolic rate was +5 per cent. The six-hour thyroidal radioiodine uptake was 88 per cent after propylthiouracil had been withheld for two days. The serum protein-bound iodine concentration was 4.0 μg. per 100 ml. It was believed that the patient was not hyperthyroid clinically.

Section of the pituitary stalk was advised because of the severe progressive exophthalmos. *On October 23, 1957, the pituitary stalk was sectioned and cauterized.* The two cut ends of the stalk were separated by a circular piece of tantalum foil placed over the diaphragma sellae.

Cortisone acetate was given postoperatively; the dosage was reduced gradually and the drug was discontinued eight days postoperatively. The patient was discharged from the hospital on the fifteenth postoperative day, with no medication. Proptosis at that time measured 31 mm. O.U. After surgery, symptoms of mild diabetes insipidus developed, and they persisted for about six weeks.

The patient was examined again on January 2, 1958. No menstruation had occurred since her operation. Previously, menses had been regular. She was weak and had generalized myalgia. There was marked loss of axillary and pubic hair. The hair on her scalp was regrowing slowly. She had lost all sexual libido. The BMR was +9 per cent. The thyroidal radioiodine uptake was 52 per cent in twenty-four hours. The serum concentration of PBI was 9.6 μg. per 100 ml., of sodium 147 mEq. per liter, and of potassium 4.8 mEq. per liter. Urinary gonadotropin by the infant-mouse method was less than 13 mouse units in twenty-four hours. Proptosis measured 31 mm. O.U. It was believed that she did not have clinical hyperthyroidism, but that adrenal and ovarian failure were developing. She was treated with hydrocortisone, 10 mg. daily, and with methyltestosterone, 5.0 mg. daily.

She was examined again on April 10, 1958. Sexual libido still was absent. Pubic hair remained decreased to about a quarter of the normal amount, and axillary hair was almost absent. The BMR was +27 per cent; the pulse rate was 92 at rest. The thyroidal radioiodine uptake was 50 per cent in five and a half hours and 59 per cent in twenty-eight hours. The serum concentration of PBI was 4.8 μg. per 100 ml., of sodium 147 mEq. per liter and of potassium 4.8 mEq. per liter. The cells seen in a vaginal smear were of the castrate type. Proptosis measured 30.5 mm. O.D. and 31.0 mm. O.S. Urinary 24-hour excretion of 17-ketosteroids (9) was 7.5 mg., and of 17-hydroxycorticosteroids (10), 3.1 mg. Gonadotropin excretion was again less than 13 mouse units per twenty-

four hours. Because it was believed that clinical hyperthyroidism was recurring, propylthiouracil in a dosage of 300 mg. daily was prescribed.

When the patient was seen again on May 7, 1958, her pulse rate was 84 and her blood pressure was 115/70 mm. Hg. The BMR had declined from the previous value of +27 per cent to a level of −3 per cent. The clinical improvement that followed the administration of propylthiouracil was convincing evidence that active hyperthyroidism had been present. The dosage of propylthiouracil was reduced to 200 mg. daily. The administration of hydrocortisone and of methyltestosterone was continued. Propylthiouracil was discontinued on July 28 and hydrocortisone and methyltestosterone were discontinued on August 12.

The patient was examined again on August 18, 1958. The BMR's were +23 and +27 per cent, and the pulse rate was 92 at rest. The six-hour thyroidal radioiodine uptake was 68 per cent and the serum PBI concentration was 5.4 μg. per 100 ml. The 24-hour excretion of urinary 17-ketosteroids was 6.0 mg., and of 17-hydroxycorticosteroids, 5.3 mg. The serum potassium concentration was 4.7 mEq. per liter. The cells seen in a vaginal smear were castrate in type. Proptosis measured 29 mm. O.D. and 31 mm. O.S. The patient noted that one week after she stopped taking propylthiouracil she suffered from heat intolerance and warm, sweaty hands. There was some stiffness of joints and muscles. On clinical grounds the patient had active hyperthyroidism again. The elevated BMR, the thyroidal radioiodine uptake value, and the response to propylthiouracil supported this belief, although the serum PBI concentration did not. Nevertheless, on August 20 she was treated with 4 mc. of radioiodine.

She was examined again on October 22, 1958. There were marked changes: her skin was cool and dry, and its texture was normal; she complained somewhat about the cold; the pulse rate was 60; the BMR was −7 per cent; and the thyroidal radioiodine uptake was 6 per cent in six hours. It was thought that she might have mild hypothyroidism. The 24-hour urinary excretion of 17-ketosteroids was 2.5 mg., and of 17-hydroxycorticosteroids, 2.1 mg.

Bioassay of serum for thyrotropin on August 18, 1958, (using McKenzie's (11) modification of the method of Adams and Purves) showed no response at two hours, but a delayed response of 144 per cent at nine hours. A two-hour response would have been typical of a thyrotropin injection of 0.15 m.u. of the U.S.P. reference standard material. The increase only at nine hours is the response previously demonstrated to exist in hyperthyroidism (12).

The patient was examined again on March 31, 1959. She was taking no medication. She had fatigue, aching muscles, and stiffness of the joints. She lacked energy. No symptoms of hypoglycemia were noted. Mild intolerance to cold was present. The blood pressure was 118/70 mm. Hg, and the pulse rate 76. The skin was dry and cool, but its texture was normal. The vagina was dry and the cervix atrophic. Deep tendon reflexes were active, with rapid return. The Power-Kepler index was positive, being 7.3. The BMR was +9 per cent. The thyroidal radioiodine uptake was 21 per cent in six and a half hours. The 24-hour urinary excretion of 17-ketosteroids was 4.9 mg., and of 17-hydroxycorticosteroids, 2.2 mg. The patient was given 25 units of ACTH intravenously over a six-hour period. The 24-hour excretion of 17-ketosteroids rose to 10.2 mg., and of 17-hydroxycorticosteroids to 13.8 mg. An assay of serum for thyrotropin again showed no significant response (114 per cent) at two hours, but a delayed response of 152 per cent at nine hours. Exophthalmos measured 30.5 mm. O.D. and 31 mm. O.S. The edema of the eyelids and the congestion of the temporal conjunctival vessels had almost completely disappeared. Excursions of the eyes were normal.

In October 1959, the situation was little different. Eye measurements were 30 mm.

O.U., the serum PBI level was 7.1 μg. per 100 ml., and the BMR was ±0 per cent. It was believed that the patient was euthyroid, but exhibited definite evidence of adrenal and ovarian failure.

DISCUSSION

The balance of evidence from the data supports the contention that hyperthyroidism occurred and persisted in the face of anterior pituitary failure. The patient appeared to be free of hyperthyroidism for a time after section of the pituitary stalk, but hyperthyroidism recurred about six months later. The delayed response observed in the bioassay of her serum for thyrotropin, and the lack of significant improvement in her eye signs suggest that the disease may still be active.

Whether or not, in our patient, the secretion of thyrotropin by the cells of the anterior lobe of the pituitary gland was depressed remains unproved; it is difficult to accept as likely the possibility that cells of the anterior lobe could, under these circumstances, be producing a marked excess of thyrotropin. The assays of serum for thyrotropin failed to disclose the type of response that is seen in myxedema (11) but did show the delayed response found in other patients with hyperthyroidism (12). Possible explanations for these results include: (a) that the activity measured in the patient's serum was an artifact in the assay (an explanation which recent evidence would tend to refute) (13); (b) that surviving pituitary cells secreted the thyroid activator measured in the assay; or (c) that a substance of extrapituitary origin had throughout been acting on the thyroid to produce hyperthyroidism.

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Letters to the Editor

THE DURATION OF INCREASED SERUM IODINE CONCENTRATION AFTER INGESTION OF BUNAMIODYL (ORABILEX)

To the Editor:

The contrast media used in radiography are the most commonly encountered substances which artifactually increase the concentration of protein-bound iodine (PBI) in serum. These compounds contain organic iodine which is determined directly as protein-bound iodine, and spuriously high PBI values thus obtained may persist for three months or longer (1). Since determination of the concentration of serum protein-bound iodine is probably the most accurate test of thyroid function, it is helpful to know the length of time an administered compound is likely to affect this laboratory test. The contrast medium, 3 (3-butyrylamino-2,4,6-triiodophenyl)-2-ethyl sodium acrylate, called bunamiodyl or Orabilex,[1] contains 57 per cent iodine (2.6 Gm. per dose), and is often used for cholecystography. The study to be described was made to ascertain the length of time this compound increases the levels of protein-bound iodine and total iodine in the serum of normal persons.

Material and methods

Three normal men ingested the usual dose of 4.5 Gm. of Orabilex and blood specimens were taken from each subject at intervals ranging from one to sixty-four days. The concentrations of serum protein-bound iodine and total iodine were determined by a modification of the method of Zak and co-workers (2). The excretion of iodine was not studied.

Results

The serum protein-bound iodine concentration had returned to normal within forty-five days in 2 subjects and within sixty days in the third (Table 1). The unbound iodine level exceeded the normal range consistently for only five days after ingestion of Orabilex. A semilogarithmic plot of all serum protein-bound iodine values (Fig. 1) yielded a curve with a steep slope at first and a gradual transition to a straight line of low slope. A straight line was fitted to the majority of the values and was extended to

[1] E. Fougera and Company, Hicksville, New York.

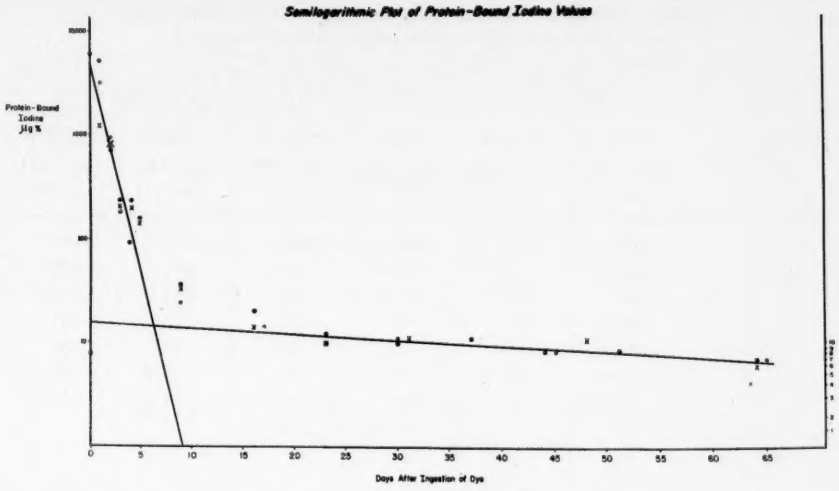

FIGURE 1

the ordinate; the steep initial slope was then obtained by subtracting in succession the straight line plots from the values remaining.

Discussion

The normal range for the concentration of protein-bound iodine in the serum is 4.0 to 8.5 µg. per 100 ml. Values in excess of 8.5 µg. per 100 ml. are found in normal persons when exogenous iodine is present. The level of total iodine in the serum, which is usually affected to a greater degree, exceeds the level of protein-bound iodine by more than 2 µg. per 100 ml. (2). Both the protein-bound iodine and the total iodine levels were greatly increased following ingestion of the contrast medium, and they fell rapidly during the first nine days. Thereafter there was slow return to normal during the next thirty-six to fifty days.

Ordinarily it is unnecessary to determine the exact value for the concentration of protein-bound iodine or total iodine in the serum when it exceeds 32 µg. per 100 ml., as concentrations above this level invariably are caused by exogenous iodine. In this study, although the unbound iodine values clearly indicated the presence of exogenous iodine during the first five days, in only 1 instance was this true when the protein-bound iodine values had fallen to less than 32 µg. per 100 ml.—the maximum sought by routine laboratory measures. The unbound iodine value, then, did not indicate the presence of exogenous iodine for longer than seventeen days after the ingestion of the contrast medium.

TABLE 1. LEVELS OF SERUM PROTEIN-BOUND IODINE AND TOTAL IODINE IN 3 SUBJECTS AFTER INGESTION OF ORABILEX

Days after ingestion	Subject No. 1		Subject No. 2		Subject No. 3	
	PBI (μg./100 ml.)	Total I (μg./100 ml.)	PBI (μg./100 ml.)	Total I (μg./100 ml.)	PBI (μg./100 ml.)	Total I (μg./100 ml.)
1	5400	6400	3400	4400	1260	1400
2	720	840	940	1420	800	920
3	240	870	210	540	230	580
4	240	870	92	172	212	344
5	154	168	72	116	140	180
9	34.4	36.8	24	24	33.6	38.0
16	20.0	21.6	—	—	14.0	16.8
17	—	—	14.4	14.4	—	—
23	10.8	13.6	—	—	10.0	10.4
29	—	—	—	—	—	—
30	10.0	11.6	10.9	10.9	—	—
31	—	—	—	—	10.8	10.8
37	10.9	11.8	—	—	—	—
43	—	—	—	—	—	—
44	8.2	8.8	—	—	—	—
45	—	—	8.0	8.5	—	—
48	—	—	—	—	10.3	10.8
51	8.0	9.4	—	—	—	—
59	—	—	—	—	6.0	6.2
64	6.6	6.6	6.8	7.4	—	—

The manufacturer states that 70 per cent of Orabilex is excreted by the kidney. The initial rapid fall of the spuriously high levels of serum protein-bound iodine and total iodine may reflect this and/or other modes of excretion; the slower fall of the serum levels to normal probably indicates the elimination by unknown mechanisms of an iodinated compound which has become "bound" or associated with serum protein.

In summary, Orabilex artifactually increased the concentrations of serum protein-bound iodine and total iodine for a maximum of fifty-nine days in 3 normal men.

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BIOPSY OF THE THYROID IN NONGOITROUS MYXEDEMA

To the Editor:

The etiology and pathogenesis of so-called spontaneous adult myxedema remains generally obscure. Although the laboratory diagnosis of hypothyroidism has been improved in recent years through the use of various tests, such as the serum protein-bound iodine (PBI) determination and studies with radioactive iodine (I^{131}), these tests do not reveal the fundamental nature of the thyroid lesion. In primary spontaneous myxedema of the adult, the finding of a diffusely enlarged, rubbery thyroid gland suggests the diagnosis of Hashimoto's chronic thyroiditis (lymphadenoid goiter) as the underlying lesion (1). An immunologic mechanism has been implicated recently in the latter condition (2). On the other hand, the absence of goiter is usually taken to indicate "primary" thyroid atrophy of unknown cause.

In cases of suspected "primary" thyroid atrophy no morphologic evidence of such atrophy is usually provided, since as a rule needle biopsy (3) is not performed in nongoitrous patients, and even in cases of goiter the material obtained by this procedure is not infrequently insufficient for an accurate diagnosis (4).

In cases of nongoitrous myxedema, however, histologic examination of thyroid tissue may provide unsuspected findings, and show lesions which differ from those of "primary" atrophy. The biopsy specimen can be obtained under local anesthesia, through a small incision (2–3 cm.) on the anterior aspect of the neck over the thyroid isthmus. This simple procedure causes very little discomfort for the patient, and it is possible to obtain a fragment of thyroid tissue large enough for a correct histologic diagnosis.

We have performed thyroid biopsies in 4 typical cases of adult nongoitrous primary myxedema. The clinical and laboratory findings are sum-

TABLE 1

	Case 1	Case 2	Case 3	Case 4
Sex	male	male	male	female
Age (yrs.)	39	23	39	64
Duration of symptoms (yrs.)	3	1	½	2
Thyroid gland	? slightly palpable	not palpable	not palpable	not palpable
B.M.R. (%) 1st determination 2nd determination	−33	−32 −25	−20 −35	− 6 −19
Serum cholesterol (mg./100 ml.)	588	425	175	296
Serum PBI (μg./100 ml.)	2.6	1.5	1.8	—
Thyroidal 24-hour I^{131} uptake (% dose)	18	11	14	10
Conversion ratio (%) (24 hrs.)	12	—	9	8
Salivary radioactivity (c.p.m./3 ml.) (24 hrs. after a dose of 50 μc.)	48,812	—	95,312	84,312
Cutaneous reaction with saline extract of thyroid	negative	negative	negative	negative
Histologic diagnosis	Hashimoto's thyroiditis (Fig. 1)	Hashimoto's thyroiditis	slight fibrosis and atrophy	marked fibrosis and atrophy (Fig. 2)

marized in Table 1. The 2 cases of Hashimoto's disease (Cases 1 and 2) were not suspected clinically; the patients were males (in whom this disease is extremely rare (5)), without any neck discomfort or evidence of thyroid enlargement. In both instances the clinical picture developed insidiously with the characteristics of "primary" thyroid atrophy. Results of the cutaneous test with saline extract of thyroid (6) were negative, but it has been shown that antibodies disappear in these cases when myxedema is well developed (7). The diagnosis of Hashimoto's disease in cases like those reported here can be made only by histologic study of the thyroid tissue (Fig. 1). The

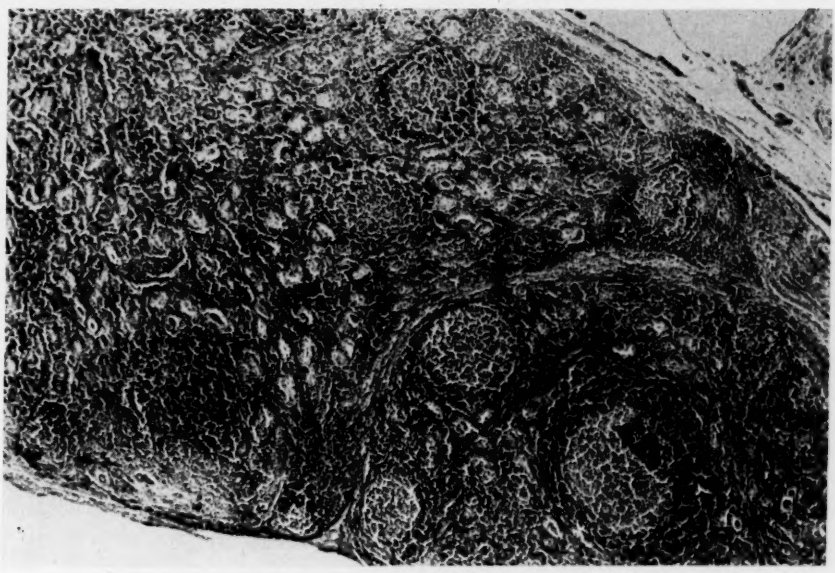

FIG. 1. *Case 1.* Hashimoto's chronic thyroiditis (reduced from ×100).

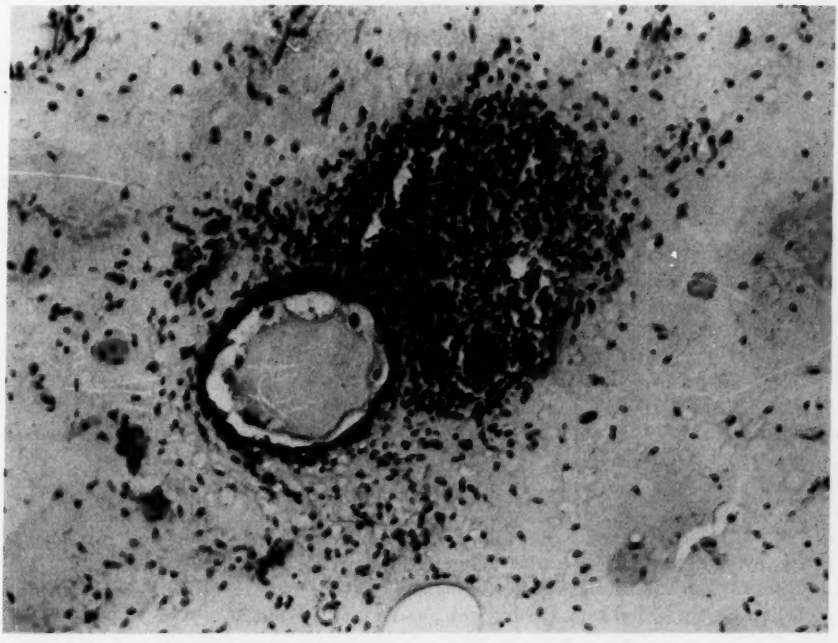

FIG. 2. *Case 4.* Fibrotic lesion of the thyroid gland with marked parenchymal atrophy and moderate lymphocytic infiltration (reduced from ×450).

absence of goiter makes a needle biopsy extremely difficult or impossible so that the only way of making a correct diagnosis is by using a technique like the one proposed here.

In Cases 3 and 4, clinically indistinguishable from the other two, the histologic examination of the thyroid showed fibrosis and parenchymal atrophy. This was more marked in the thyroid of Case 4, which also presented a few localized areas of lymphocytic infiltration (Fig. 2). It is possible that the histologic picture observed in Cases 3 and 4 agrees with that seen at autopsy (8) in "primary" thyroid atrophy, and could be the final result of an inflammatory process in the gland. It is also very similar to the histologic picture described by Hedinger (9) as "chronic atrophic thyroiditis." Whether Hashimoto's disease itself can end up as a fibrotic lesion is a moot question, which might possibly be answered with the help of serial biopsies.

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January 16, 1960

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The Endocrine Society

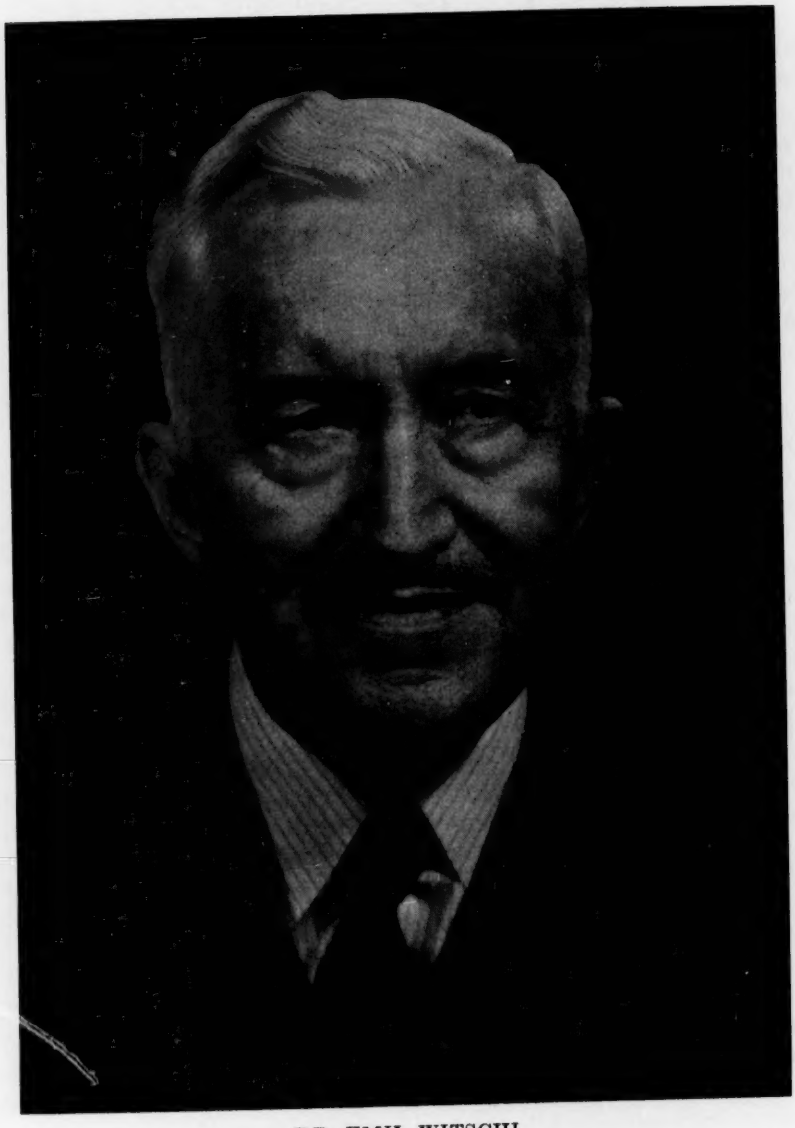
Recipients of the 1960 Awards

THE FRED CONRAD KOCH AWARD OF THE ENDOCRINE SOCIETY

The Fred Conrad Koch Award is the honor of highest distinction conferred by The Endocrine Society. In presenting this medal to *Professor Emil Witschi,* the Society pays tribute to a man who is both a pioneer and active contributor to the field of sex embryology and endocrinology.

Born in an Alpine valley near Bern, Switzerland, Emil Witschi developed early a fascination for the variety of animals with whom he shared his pastoral surroundings. This interest in natural history was nurtured under the tutorial guidance of the classical embryologist, Richard Hertwig, with whom Professor Witschi prepared for his doctorate, awarded by the University of Munich in 1913. His indoctrination as a naturalist and his recognition of the importance of comparative biology in the understanding of embryologic and physiologic events have led him to range broadly in his investigations of gonadal and other endocrinologic problems. Contained in his abundant scientific bibliography are studies on more than 30 different animal forms, ranging from invertebrates to man and including such unusual creatures as the Western banded gecko, African bishop birds, the nine-banded Armadillo and the diamond-back terrapin. Few learned societies have the privilege of honoring a colleague with achievements so diversified as to include the identification of a sub-species of frog, a description of ear development for underwater hearing, the analysis of nuptial plumage in African birds, and the study of sex behavior of herring gulls. The broad scope of his work is, itself, a tribute to his zealous scientific curiosity, pursued with endless vitality and with the highest degree of competence.

The name of Witschi is identified most intimately with the inductor theory of sex differentiation, formulated and proposed by him in 1914. In those early years his studies on the process of sex differentiation in *Rana temporaria* led him to the realization that all embryos and primordial germ cells are bipotential and that several factors, both genetic and non-genetic, decide the alternative of male or female differentiation. He demonstrated that the embryonic gonadal cortex is an inductor of female differentiation, and the medulla an inductor of male differentiation. These concepts, for-



DR. EMIL WITSCHI

mulated by Witschi before 1920, serve as a basis for the current re-evaluation of human intersexuality—a development in which he has played an active role.

In 1926 Professor Witschi took leave of his native Switzerland and his post as a lecturer at the University of Basel to visit the United States as a Rockefeller Foundation Fellow. He spent nearly two years in the Osborn laboratory at Yale, and with Carl Moore at Chicago and Herbert Evans at California. In each laboratory he found a stimulating environment and a great interest in the field of sex differentiation. With the emphasis on this area of research shifting from Europe to the United States, the decision to return to this country and assume American citizenship was a natural one for Professor Witschi and in this he was supported by his faithful wife Martha. That he has been forgiven his exodus from the Swiss scientific family is evidenced by his appointment, nearly twenty years ago, to the Swiss Academy of Sciences and by the honorary Doctor of Medicine degree conferred on him by the University of Basel on the occasion of the 500th anniversary of that historic institution.

In America, Professor Witschi chose the beautiful setting of the State University of Iowa, located on the fertile rolling hills overlooking the Iowa river. His appointment in 1927 as Professor of Zoology was destined to add further luster to, and to maintain the prominence in endocrine research of the department led by W. W. Swingle. Continuously, Professor Witschi and his growing school of Iowa students broadened the attack on the problem of sex differentiation to include the study of fishes, reptiles, birds and mammals. As a result, Witschi's inductor theory has found widespread acceptance as a fundamental principle in the development of all vertebrate gonads. Through the years, he has developed a truly remarkable laboratory for studies in comparative endocrinology. With these facilities it has been possible to study many hormonally controlled sexual characteristics that extend beyond those found in the usual species of laboratory animals. His studies on the comparative aspects of pituitary gonadotropins provide important fundamentals for the current activity in this field. His analysis of the hormonal control of feather and bill coloration in birds reveals basic considerations bearing on the evolution of endocrine reactions. His work on temperature effects and over-ripeness in amphibian eggs has been of fundamental importance in the fields of teratogenesis and fetal abnormalities. Among his numerous contributions to the study of genetics is the discovery of the chromosomal mechanisms for sex inheritance in two different species of amphibia.

Throughout this lifetime of devotion to scientific research, Emil Witschi has fulfilled with great conscience his responsibilities as a member of the teaching faculty of a great State University. In 1955 he published his

second book, an outstanding textbook entitled *The Development of Vertebrates.* Replete with original concepts and data, it represents an important contribution to the teaching of embryology. The first book under Witschi's authorship, also prepared for students, appeared nearly 40 years earlier when, as a teacher in the Science Gymnasium of Basel, he wrote and illustrated a delightful volume entitled *The Land Animals.* Intended to inspire in his young students an interest in biology, this book served also as an outlet for his artistic inclinations. With this volume, Witschi purged himself of a lingering idea to forsake science in favor of a career in the graphic arts. Those who have observed his classroom lectures, which might well serve as sketching lessions in a school of fine arts, will recognize that he has succeeded in pursuing both courses.

By virtue of the achievements that have been cited and numerous others that are omitted here for the sake of brevity, Emil Witschi is amply qualified to receive this coveted award. Not to be omitted, however, is recognition of his invaluable service to the field of endocrinology through the inspiration he has provided to young scientists. He has advised and guided over forty men and women to the Ph.D. degree, and many other young investigators whose researches border on his interests have profited from his consultation, encouragement and suggestions. This boundless fervor to broaden the horizon of scientific understanding characterizes Emil Witschi. His continuing activity in research and teaching will contribute toward a further expansion of this horizon.

THE CIBA AWARD

DR. DON HARRY NELSON

Dr. Don Harry Nelson is the recipient of the Ciba Award for 1960, presented by The Endocrine Society in acknowledgment of his distinguished contributions to man's knowledge of the functions of the adrenal cortex. The precision and incisiveness of these highly original studies have established him as the one of most gifted young investigators in endocrinology.

He has provided a chemical technique for the direct study of the circulating adrenal hormones and a method for obtaining hormone-rich blood directly from the adrenal gland, and has shown that the hormone content of such blood collected close to the gland greatly exceeds that in the peripheral blood. This finding alters our concept of the "milieu intérieur" as a homogenous environment and shows that this internal environment has within it many

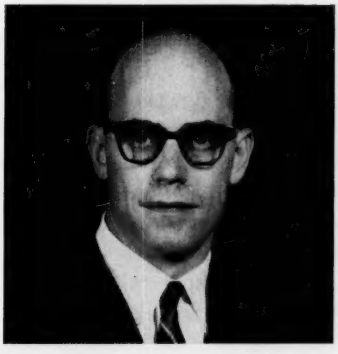
internal pools and eddies. A method developed by Dr. Nelson permits assessment of the amount of adrenocorticotropic hormone in peripheral blood, thus adding much to our ability to analyze the basic mechanisms involved in disease states affecting the pituitary-adrenal axis. He has participated in vital phases of the earliest studies on the nonhormonal agents capable of suppressing adrenocortical function in animals and man. He has described the development of pituitary tumors in previously adrenalectomized patients and has characterized their endocrine and metabolic effects upon the host.

"Careful observer, able investigator, competent physician and amiable colleague, The Endocrine Society hails and commends you in affection and esteem."

THE SQUIBB FELLOWSHIP

No qualified candidates were available for the 1960 Squibb Fellowship. The Awards Committee, with the permission of the donors, decided to use the Fellowship funds for additional travel grants to the First International Congress of Endocrinology.

THE UPJOHN SCHOLAR



DR. CHARLES R. KLEEMAN

The Upjohn Scholar for 1960 is *Dr. Charles R. Kleeman.* Dr. Kleeman was born in Los Angeles, California on August 19, 1923. He obtained his collegiate training at the University of California, and was granted the B.S. degree from that institution in 1944, and the M.D. from the University of California School of Medicine in 1947. Dr. Kleeman has held the following positions: Intern, University of California Service, San Francisco Hospital 1947–48; Assistant Resident in Pathology, Mallory Institute, Boston City Hospital, 1948–49; Intermediate Resident in Medicine, Newington Veterans Administration Hospital, and Fellow in Metabolism, Yale University 1950–51.

Associate Chief, Biochemistry Unit, Q.M. Climatic Research Laboratory, Lawrence, Massachusetts, 1951–53; Lieutenant, Medical Corps, U. S. Army, 1951–53; Instructor in Medicine, 1953–55, Assistant Professor 1955–56, Metabolic Section, Department of Medicine, Yale University School of Medicine; Associate Clinical Professor of Medicine,

University of California Medical School, Los Angeles, 1956–58; Chief, Metabolic Section, Veterans Administration Hospital, Los Angeles, California, 1956 to the present; Associate Professor of Medicine, University of California Medical Center, Los Angeles, 1959 to the present.

During the last ten years Dr. Kleeman has published more than fifty reports concerned with the role of the thyroid, parathyroids, adrenal cortex and neurohypophysis in controlling water and electrolyte metabolism in human beings and experimental animals.

During the year beginning July 1960, Dr. Kleeman will be on sabbatical leave. He will continue his investigations on water metabolism in the laboratory of Dr. Hugh Dawson, Department of Physiology, University College, London, England. The Upjohn Scholarship will be used by Dr. Kleeman for travel, and for support of his research program in England.

* * *

PRESENTATION TO DR. ROY GRAHAM HOSKINS

This presentation is made to *Dr. Roy Graham Hoskins* in recognition of his outstanding contributions to the advancement and dissemination of knowledge of the glands of internal secretion and in appreciation of his great labors on behalf of The Endocrine Society.

A pioneer in the unfolding role of the endocrines in man's physical and mental well-being, he helped found a science and give substance to a bright new epic in medicine. The sound guidance he gave to this field, especially during its formative period, was of crucial importance. To these endeavors he brought a keen and critical mind, an indomitable courage, boundless energy, a cheerful spirit and farsighted wisdom.

Roy Hoskins rallied interest to form in 1917 The Association for the Study of Internal Secretions, later to become The Endocrine Society. In the course of long and invaluable service to this Society he gave helmsmanship to its destinies as president, councilor and committeeman. He ventured the inauguration of a new journal, *Endocrinology*, serving as its editor for twenty-five years, and, in addition, significantly influenced the founding of *The Journal of Clinical Endocrinology*. Striving skillfully and often singlehandedly against lingering perversities, he handed on to future generations of endocrinologists two journals of distinction in the world of science and medicine.

To Roy G. Hoskins, who has served his field faithfully and well as scientist, author, editor, historian, teacher, friend and wise counselor, The Endocrine Society hereby does honor, proudly and with enduring gratitude.

The 1961 Annual Meeting

The Forty-Third Annual Meeting of the Endocrine Society will be held in the Hotel Biltmore, New York, N. Y., Thursday, Friday and Saturday, *June 22, 23 and 24, 1961.*

The Chairman of Local Arrangements is Dr. Joseph W. Jailer.

The Scientific Sessions will be held from 9:00 a.m. to 5:00 p.m. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 23, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make their hotel reservations immediately. The Biltmore will hold 300 bedrooms for members until May 1, 1961, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 26 for the American Medical Association and other meetings, it is imperative that your reservations be made from June 26 on directly through the A. M. A. Housing Bureau. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1961.

Final program, membership card and advance registration forms will be sent on May 1, 1961 to members whose current dues have been paid.

The 1961 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Twelfth Postgraduate Assembly in Endocrinology and Metabolism

Under the Co-Sponsorship of the Endocrine Society and the University of Wisconsin Medical School

Madison, Wisconsin

October 24 through 28, 1960

For further information, please write to the local chairman, Dr. Edgar S. Gordon, The University Hospitals, 1300 University Avenue, Madison 6, Wisconsin. The fee is $100.00 for physicians and $30.00 for Residents or Fellows taking the course.

Acknowledgment

The Editorial Board of The Journal of Clinical Endocrinology and Metabolism wishes to acknowledge with gratitude the generous and expert aid of the following reviewers:

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Editor-in-Chief

Symposium on Recent Developments in Research Methods and Instrumentation

The 10th Annual Instrument Symposium and Research Equipment Exhibit will be held *October 4–7, 1960*, at the National Institutes of Health, Bethesda, Maryland. Primary topics for discussion on the scientific program include fluorescence, infra-red, activation analysis, ultracentrifuge, microscopy and electrodes. Examples of the latest types of research equipment will be exhibited by instrument manufacturers. Exhibit Manager—Mr. James B. Davis, National Institutes of Health Bethesda 14, Maryland. Phone: OLiver 6-4000, Extension 2315.

Purified Radioactive Steroids Available

The Endocrinology Study Section, National Institutes of Health, has the following highly purified radioactive steroids available for distribution free to qualified investigators:

Material	*Specific Activity*
7-^{3}H Aldosterone	20 μc./μg.
7-^{3}H Cortisol	28 μc./μg.

Workers may obtain supplies of these steroids by writing to Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland, describing briefly the proposed work and giving an estimate of the amount of steroids required. Requests are filled as promptly as possible after review by a subcommittee. These steroids are prepared and distributed under a program generously supported by the National Institutes of Health.

These materials are stored at Tracerlab, Inc., Waltham, Massachusetts. They are dispensed in sealed glass ampules containing 30 μc. each, and in a solution of benzene and methanol.

Shipments will be made from Tracerlab, Inc., and the recipient will be billed by them, at the rate of $5.00 per ampule for handling and mailing.